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REGIONAL MELANISM IN AQUATIC BEETLES[1]

FRANK N. YOUNG

Indiana University, Bloomington, Ind.

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The tendency for insects in certain regions to be darker in color than their relatives elsewhere was first pointed out by H. W. Bates among the butterflies of the Amazonas. Similar tendencies to regional melanism have since been noted in other regions, but the biological basis of the condition is still a matter of surmise except in a few cases which have been investigated in detail. The most thoroughly studied example of regional melanism is that of the moths of the industrial midlands of England. Here about 70 species are now known to have melanic mutant forms which are in the process of replacing the normal light forms of the species Kettlewell (1958) and others have shown that this "industrial melanism" is a dramatic illustration of natural selection acting upon genetic mutations.

In most cases, however, the darkened condition has been tacitly attributed to the action of environment. There is direct experimental evidence that low temperatures favor melanin formation in insects, but evidence that humidity alone has the same direct effect is mostly circumstantial. Among the Coleoptera, the dark color patterns found frequently in humid regions (or even humid-cold areas) have proven in many instances to be genetically controlled, and detailed studies in areas where a number of species show unusual darkening should further understanding of the interactions between genetic and environmental factors.

In many insects, particularly aquatic beetles, increased melanism is hard to detect because it cannot be visually distinguished from the normally dark condition. The biochemical pathways by which tyrosine or other precursors convert to melanin are deeply involved with the whole metabolism. It is even possible that respiratory enzymes necessary in the larval stages of aquatic forms are "excreted" as melanin in the cuticle by the adults. In any case, melanism is often the normal condition, and it is only in areas where a general lightening of the color of many species has taken place that a secondary darkening of the pattern can be distinguished.

In Peninsular Florida melanism in a number of species of water beetles is

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conspicuous because the dark forms contrast strikingly with members of the same or related species in the coastal plain to the north or the Antilles to the south (Young, 1954). The darkening is of very different degree in different groups and species and seems to be due to at least two distinct processes: (1) An increase of diffuse melanization in or on the light portions of the pattern, and (2) An extension of the dark elements of the basic color pattern. The first of these effects may be entirely environmental and has been observed in other areas of the world, particularly in very humid regions, but the changes in the dark patterns parallel genetically controlled differences in other beetles.

The populations of melanic forms in Peninsular Florida are, in part, continuous with populations of the same species in the inner Coastal Plain and Piedmont. *Coptotomus interrogatus*, *Bidessus pullus*, and *Tropisternus mexicanus* are examples of water beetles which have dark forms in Florida. In these the Florida forms intergrade with the lighter forms in a relatively narrow zone running roughly from western Florida and southern Alabama across the southern counties of Georgia. This zone does not seem to be more than a hundred miles wide, and the greatest width is around the conjunction of Apalachicola, Flint, and Chattahoochee rivers.

In other cases, for example *Matus ovatus* and *Bidessus fuscatus*, there is a zone across western Florida, southern Alabama, Georgia, and South Carolina which seems to be unoccupied by any populations of the species. This apparent "dead zone" is probably a product of inadequate collecting, but its appearance in the ranges of some other insects (for example leaf hoppers) suggests that it is due to the great contrast in environments between the inner Coastal Plain-Piedmont strip and the outer Coastal Plain which includes all of Florida.

The following table presents a series of Floridian water beetles, selected to show the range of relationships over which melanism occurs. The degree of darkening is correlated with the closeness

Some water beetles which show increased melanism in peninsular Florida

Complex or related species in Eastern U. S. or Antilles	Florida form showing melanism	Degree of melanism of Florida form	Taxonomic relationships
Hydrocanthus iricolor Say	*H. regius* Young	Intensely black	Distinct species
Matus ovatus ovatus Leech	M. o. *blatchleyi* Leech	Intense; almost black	Possibly distinct species
Tropisternus mexicanus striolatus (LeC.)	*T. m. viridis* Young & Spangler	Intense; dark pattern greatly expanded	Subspecies
Coptotomus interrogatus interrogatus (Fabr.)	*C. i. obscurus* Sharp	Fairly intense; dark pattern expanded	Subspecies
Bidessus pullus pullus (LeC.)	*B. p. floridanus* Fall	Fairly intense; dark pattern expanded	Subspecies
Bidessus fuscatus (Crotch)	*B. fuscatus* subsp.?	Moderately intense; dark pattern expanded	Probably distinct subspecies
Enochrus pygmaeus complex	*E. pygmaeus* subsp.?	Moderately intense	Possibly distinct subspecies
Enochrus ochraceus (Melsh.)	*E. ochraceus* (Melsh.)	Moderately intense	Identical forms
Berosus infuscatus (LeC.)	*B. infuscatus* (LeC.)	Moderately intense	Identical forms
Laccophilus proximus Say	*L. proximus* Say	Variable; specimens usually darker	Identical forms

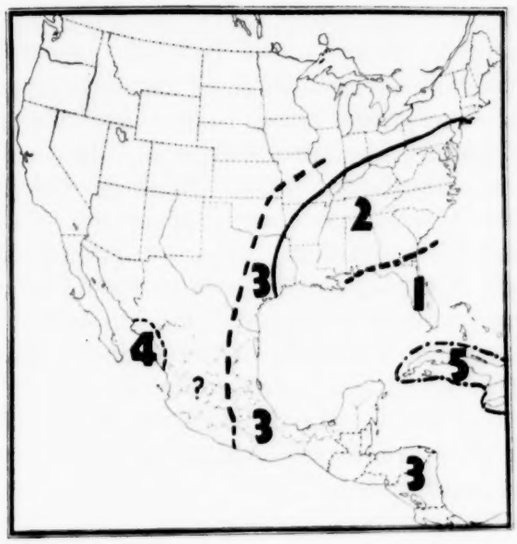

FIG. 1. Approximate distribution of members of the *Tropisternus mexicanus* complex: (1) *T. m. viridis;* (2) *striolatus;* (3) *mexicanus;* (4) unnamed; (5) *proximus.* Uncertain relationships are indicated by question marks.

of relationship, adjudged on the basis of other morphological characters. That is, morphologically distinct, allopatric forms tend to be very dark while the peninsular Florida populations of widespread species such as *Laccophilus proximus* are not much darker than those from other regions. Over 30 species of the families Dytiscidae, Haliplidae, Noteridae, and Hydrophilidae show some degree of melanism in comparison with their closest relatives outside this region. This represents about 40% or more of the common species (about 75) which occur in the lowland (pine flatwoods) situations in the peninsula.

It is not possible at present to generalize all these cases because most of them have not yet been studied in any detail. Several different kinds of factors may be involved although it looks as if we are dealing with a case in which regional melanism has been or is being produced by a few interrelated factors. The total situation cannot be as simple as industrial melanism because species of very different age (in the sense of regional occupancy) are involved. Some of the melanic forms in Florida may be relics of pre-ice age conditions; others may have arisen in the last few decades.

Melanism in the Floridian subspecies of *Tropisternus mexicanus* (Castelnau) has been intensively studied in recent years. The species ranges from Central America into the eastern United States. In different areas of this range different color patterns predominate. The distribution of these is shown in the accompanying map (fig. 1), and illustrated in figure 2.

The typical pattern (in taxonomic sense) predominates in an area which extends roughly from Panama to Missouri, continuously over nearly all of Central America and southern Mexico, but is restricted to the lower elevations along the east coast in northern Mexico and from there extends as a narrow tongue into the United States. The dark elements of the typical pattern are expanded in specimens from most of the eastern United States (*striolatus* LeC.), and in peninsular Florida the dark elements are extended to such an extent that in most individuals pale areas are almost lacking (*viridis* Young and Spangler). Two other patterns, both based on reduction of the dark areas of the typical pattern appear in western Mexico (unnamed) and in Cuba (*proximus* Sharp).

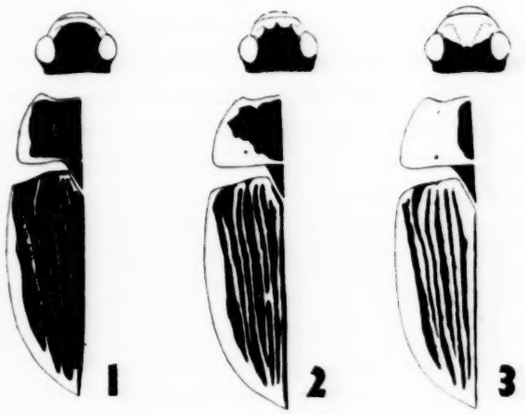

FIG. 2. Semidiagrammatic drawings of (1) *Tropisternus mexicanus viridis,* (2) *T. m. striolatus,* and (3) *T. m. mexicanus,* to show color patterns of head, pronotum, and elytron.

The typical pattern and the two patterns in the eastern United States are thought to be due to genetic rather than purely environmental influence for the following reasons. (1) The patterns are generally uniform over extensive areas within which environmental conditions vary. (2) Intermediates between the patterns occur in definite zones between areas of general uniformity and not at random. (3) The intermediates seem to represent step-like stages between patterns rather than a continuous spectrum. (4) Similar differences in patterns in other beetles have been proven to be genetic. (5) Other species of *Tropisternus* show similar patterns in some parts of their ranges, but there is no concordance with *T. mexicanus*.

The preceding statements are largely based on an extensive study of the variation in *Tropisternus mexicanus* and related forms. The details of this study will be reported elsewhere, but the following summaries may help to clarify the points.

1. The general uniformity of the basic color pattern over extensive areas has been demonstrated in large series from throughout the range of the species. The three patterns shown in figure 2 and the intermediate patterns between them were rated on separate scales for head, pronotum, and elytra for approximately 500 specimens from Florida, 700 from the eastern United States other than Florida, and 500 from Costa Rica, Nicaragua, Panama, Guatemala, and Mexico. As an example of the results of these comparisons, the pronotal patterns which show the most striking differences between areas also show considerable constancy within the areas. The pronotal pattern of 1 in figure 2 occurs in over 50% of all specimens seen from Peninsular Florida south of a line running roughly from Perry to Jacksonville, and the remainder of the specimens examined lie between 1 and 2 in pattern and only a very few had the pattern as reduced as in 2. Conversely, from southern Texas south into Central America, pattern 3 (or a more reduced pattern) occurs in almost 100% of the specimens, the exceptions being mostly individuals which are obviously deformed. In Indiana, Kentucky, Ohio, Virginia, North and South Carolina, Tennessee, Mississippi, Louisiana, most of Arkansas, and Albabama and Georgia north of a line running roughly from Mobile to Savannah, pattern 2 occurs in more than 50% of all specimens examined, and in most series exceeds 80%. The same general relationships hold in regard to the patterns of the head and elytron, but the concordance is not absolute.

2. The intermediates between patterns 1 and 2 occur roughly in a zone bounded by a line running from Mobile to Savannah, and one running from Perry to Jacksonville in Florida. Within this zone intermediates represent more than 45% of the sample of about 200 specimens. The material at hand is not adequate to determine whether a uniform cline from *viridis* (pattern 1) to *striolatus* (pattern 2) is present or whether there is a more abrupt break somewhere in southern Alabama and Georgia. Intermediates have been collected in the following counties: ALABAMA: Butler, Houston, and Mobile. FLORIDA: Bay, Calhoun, Columbia, Duval, Franklin, Gadsden, Gulf, Jackson, Leon, Liberty, Okaloosa, Taylor, Wakulla, and Walton. GEORGIA: Baker, Charlton, Colquitt, Decatur, Dougherty, Early, Liberty, Lowndes, Mitchell, Seminole, and Thomas.

Intermediates between pronotal patterns 2 and 3 have been found in Texas, Oklahoma, central Missouri, Illinois, and northwestern Arkansas. Any pattern more reduced than 2 is very rare east of the Mississippi (Florida, Georgia, Alabama, Louisiana, and Mississippi, 0 of about 600; Tennessee, 0 of 118; Indiana, 1 of 116; Ohio, 2 of 26; Illinois, 1 of 48; North Carolina, South Carolina, Virginia, 0 of about 50). West of the Mississippi, however, pattern 3 is frequent (Arkansas, 5 of 17; Missouri (Boone County), 58

of 100; Missouri (Shannon, St. Louis, Jefferson, and Giradeau counties), 3 of 86; Oklahoma and Kansas, 7 of 10; Texas, 13 of 16). Only 1 of 52 specimens from northeastern and Central Mexico has a pronotal pattern more extended than 3. In very large series from Nicaragua, and smaller series from Costa Rica, Panama, and western Mexico no specimen has a pronotal pattern more extended than 3 and in some extreme examples from Sinaloa the pronotal markings are reduced to a narrow line.

3. The intermediates between the basic patterns 1, 2, and 3 seem to be step-like rather than forming a continuum. In most series containing intermediates between patterns 1 and 2, the intermediates can be grouped into four categories. Similarly intermediates between 2 and 3 can be grouped into three categories. It is possible that the distinctiveness of the intermediate categories is an artifact of the system of rating, but the frequency distribution of different categories is not in accord with a continuous series of intermediates. For example, in a series of 100 specimens from Boone County, Missouri, 58 showed the pronotal pattern 3, 30 showed the pronotal pattern 2 and only 12 showed patterns intermediate between 2 and 3 or more reduced than 3.

4. Differences in color pattern similar to those in *Tropisternus mexicanus* occur in other beetles and are in some cases known to be genetically controlled. Dobzhansky (1951: 142–143) summarizes the data on *Harmonia axyridis*. In this lady-bird beetle various genetic races show rough correspondence to climatic areas, and in the intermediate zones the races intermingle and different phenotypes vary in frequency. (For further details see also Dobzhansky 1933; Komai, *et al.*, 1950; and Oshima, *et al.*, 1956.) In other insects some phenocopies produced by temperature changes are comparable to these genetic races, but a similar correlation with humidity does not seem to have been shown. The distribution of the forms of *T. mexicanus* is inconsistent with any simple temperature influence whether we approach it in terms of cold producing increased melanism or high temperatures decreasing melanism. Correlations of color pattern with high humidity, which could influence the beetles through the action on the terrestrial pupal stage, does not seem to be close, and as was noted earlier seems to be restricted to a diffuse melanism suffusing the light elements of the pattern. Pattern 1 (*viridis*) is not known to occur in Louisiana, for example, although the climatic conditions are very similar to those in Peninsular Florida.

5. Other species of *Tropisternus*, notably *T. lateralis* Fabricius, show differences in extent of the dark elements of the pattern in parts of their ranges. Some forms of *T. lateralis* resemble *T. mexicanus* rather closely, but there is no apparent concordance of the ranges. In fact, the striped forms of *T. lateralis* seem to inhabit mountainous regions while a uniformly dark form occurs in the dry or wet lowlands of eastern Mexico and throughout the United States including Florida.[2]

The peninsular Florida race *viridis* (Young and Spangler, 1956) is unique in being the darkest of any of the known forms of *T. mexicanus*. Its position in Florida is especially suggestive if we consider the dark pattern primitive and concur with the hypothesis of centrifugal speciation (Brown, 1957). We could postulate that the center of origin of the *mexicanus* complex was southern Mexico and that progressive waves of genetic material spread from there. The earliest occupants of the eastern United States,

[2] David P. Wooldridge at Indiana University has recently succeeded in rearing *T. lateralis* through two generations under varying conditions of humidity and temperature. We can detect no differences in the color patterns of the specimens reared under the most extreme conditions and believe that strict environmental control of the color pattern is excluded. Similar experiments on *T. mexicanus* are in progress, but have been delayed because of the failure of this species to breed under winter conditions.

if this hypothesis holds, were similar to *viridis* which represents an early modification of the primitive dark pattern. These early forms were modified by a later wave (*striolatus*) which is now being changed by a wave representing a still more reduced dark pattern (typical *mexicanus*). The Sinoloan and Cuban patterns might represent minor waves of the *mexicanus* stock which occurred during the Pleistocene when the *striolatus-viridis* section of the species was isolated by climatic conditions from the Mexican-Central American section.

Something like a wave-like progression of forms from some center in Mexico probably has occurred. The main obstacle to explaining the entire distribution on centrifugal speciation is that *viridis* does not appear to be primitive, but rather may represent a fairly recent modification of *striolatus*. The peninsular Florida population is remarkably uniform over a considerable area. It is only in western Florida, southern Alabama, and southern Georgia that we find numerous intermediates between the *viridis* pattern and *striolatus*, and these intermediates occur roughly along the border of the Sabalian biotic province. North of this rather narrow zone of integration the populations are consistently *striolatus*-like in pattern, and to the south consistently *viridis* until we reach extreme southern Florida. Here a very small percentage of specimens show a reduction of the dark portions of the pattern and resemble *striolatus*.

The appearance of *striolatus*-like forms at the extreme southern edge of the range of *viridis* in Florida suggests that the *viridis* pattern may be due to a mutation or mutations which appeared relatively recently and have not yet extended entirely over peninsular Florida nor reached to the north much beyond the limits of the Pleistocene islands.

The fact that *viridis* is part of an ecological assemblage which shows regional melanism suggests that environment is involved directly, but the sum of the evidence points, however, to genetic control of the dark coloration. Some selective action may therefore favor the dark form in Florida and in this regard we should consider the species in its normal habitats.

The typical *Tropisternus mexicanus* pattern is probably concealing in the normal stream habitats of the species. The lighter colors tend to match the light bottoms of stream bottoms in the general area of occupancy and the dark stripes serve to disrupt the general form. The *viridis* pattern, on the other hand, would furnish no protection against a light colored sand or silt bottom, but is almost undetectable on the muck and peat bottoms of many situations in Florida. In correlation with this hypothesis that a dark form is at an advantage in escaping predators under Florida conditions, *viridis* occurs in a wide variety of lenitic situations in contrast to the primarily lotic environment of the lighter forms in other regions.

If the coloration of *viridis* and other melanic species in Florida is indeed protective, similar dark forms should be found in regions where excessively poor drainage allows the accumulation of mucks and peats as in Florida. No situation exactly comparable to that in the peninsular water beetles has come to my attention, but there are indications of incipient darkening of light colored forms through diffuse melanization in some areas of Central America and in restricted areas of Brazil. This diffuse melanism does not seem to be a step toward an extension of the dark pattern, but it may in part serve the same function of concealment.

Summary

A significant number of the aquatic beetles (Coleoptera: Dytiscidae, Haliplidae, and Hydrophilidae) found in the flatwoods of Florida are much darker than their relatives in the adjacent coastal plain or in the Antilles. The Florida

race of the *Tropisternus mexicanus* complex is a striking example. Most members of this *Rassenkreis* are light colored with narrow dark stripes on the elytra, but in peninsular Florida a very dark form (*viridis*) replaces the usual light race of the eastern United States (*striolatus*). Nonconcordance with environmental conditions, the apparently step-like nature of the intermediates, and the absence of concordant variants in other members of the genus suggest genetic rather than environmental control of the patterns. The occurrence of lighter colored, *striolatus*-like, specimens in extreme southern Florida suggests that *viridis* may be a relatively recent mutant of which the genes have not yet spread through the entire Florida population. Dark forms such as *viridis* may be favored by being less visible to predators in dark-bottomed habitats such as are common in Florida. If so, the regional melanism of the water beetles may be due directly to the differential elimination of light and dark forms as in the "industrial melanism" of the moths of the English midlands.

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CYTOGENETICS OF THE GRASSHOPPER *MORABA SCURRA*. V. BIOMETRIC EFFECTS OF CHROMOSOMAL INVERSIONS

M. J. D. White and Lesley E. Andrew

Department of Zoology, University of Melbourne, Australia

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Introduction

The effects of some of the naturally occurring chromosomal inversions of the Australian grasshopper *Moraba scurra* Rehn on viability have been described in previous papers (White 1956, 1957, 1958, 1959; Lewontin and White 1960). These are pericentric inversions in two autosomes, the "CD" and "EF" elements. Most colonies of the species in the northern part of its distribution area show polymorphism for both chromosome pairs, or in some instances for the CD chromosome only. In the southern part of its range (i.e. in the state of Victoria and some adjacent areas of New South Wales) the natural populations seem to be almost or entirely monomorphic for the "Standard" sequences of both chromosomes.

Both cytological polymorphisms seem to be maintained in equilibrium, in those populations in which they occur, by heterosis. But as far as male viability is concerned, there is a powerful interaction between the two polymorphisms. Thus the genetic equilibrium is a complex one, the two heterotic systems being interlocked and not independent. Lewontin and White (1960) used an IBM digital computer to calculate the mean relative viability constants ($\overline{W}$) for populations having all combinations of chromosome frequencies. From the information so obtained it was possible to construct "topographies" for each sample, in which the vertical dimension represents mean viability, the contours being lines of equal viability. It was shown that all the topographies so obtained were of the same general type, with a steep ridge running obliquely across the surface between two adaptive peaks. The middle part of the ridge is at a lower level and constitutes a saddle or col; the existing natural populations at five collecting stations were shown to have chromosome frequencies which locate them precisely on the respective saddles. This may be merely a graphic way of stating that the two heterotic systems are to some extent subtractive rather than additive. But the topographies seem to be a useful technique of analysis. They are, of course, based on the assumption that the relative viabilities of the various genotypes are frequency-independent. In actuality we suspect that this is not so, i.e. that the genetic equilibrium depends, to some extent, on the selective value of each genotype (or some of them) declining as that genotype becomes more frequent in the population.

In an attempt to throw more light on the actual role played by these inversions in the natural populations in which they occur we have begun a series of biometrical studies in which the individual adult grasshoppers are measured in various ways before having the testis removed for cytological study.

Material and Methods

In the present paper we are concerned with the natural populations at four localities—the cemeteries at Wombat, Wallendbeen and Murrumbateman, N.S.W., and a strip of land along the railroad near Royalla, Australian Capital Territory. The latter locality (Royalla "B") is about two miles north of Royalla railroad station. A total of 3,123 adult males were studied, in 1958-1959. These were collected in the field, brought back to the

laboratory and kept in the presence of an abundant supply of food (the plant *Helichrysum apiculatum* (Labill.) DC) so as to ensure that they were fully fed. Each individual was killed in a cyanide jar and immediately weighed on a torsion balance (to the nearest half milligram). Various linear measurements were then made on the stage of a low power microscope with an ocular micrometer, and the number of spines in the inner and outer rows on the hind tibiae was counted. The testis was then removed and made into an aceto-orcein squash preparation. Such preparations, sealed with nail varnish can be kept for up to a month or more in a refrigerator without serious deterioration.

About 10% of the individuals had lost one or other hind leg as a result of autoamputation. In determining the wet weight in such cases an appropriate allowance was made, the weight of the remaining leg being included twice. Individuals that have lost two hind legs are so rare as to be almost non-existent.

In the present paper we shall only be concerned with the data on wet weight, which seems to be the most satisfactory index of overall size. The various linear measurements studied (length of head, pronotum, hind femur and subgenital plate) seem, in general, to be fairly highly correlated with the wet weight.

Since the work is relatively time-consuming, it was not possible to have all the individuals from a particular locality collected or studied on the same day or even in the same month. All the individuals were imagines, but it is probable that minor changes in weight take place during the period May-October, over which the material was studied. In the case of the Wombat colony 726 individuals were studied in 1958 and 627 in 1959, but the data of these two years do not show significant differences and have consequently been combined (table 1).

Results

The frequencies of the four chromosome sequences (Standard CD, Blundell CD, Standard EF and Tidbinbilla EF) do not seem to have changed significantly at Wombat, Wallendbeen and Royalla since 1956, when the first collections were made at those localities. At Murrumbateman there has been an apparent increase in the frequency of the Tidbinbilla sequence (from 8.48% in 1955–1956 to 11.82% in 1959), which is just significant at the 5% level. The relative viabilities of the various genotypes at all localities except Wallendbeen are similar to those determined earlier, i.e. they would yield topographies of the same general form as those previously calculated. This adds Murrumbateman to the list of localities at which the two heterotic systems are interacting in a particular manner.

The relative viabilities in the case of the Wallendbeen sample, as determined in our usual manner, namely from the ratios of numbers of observed individuals to the numbers expected on the binomial square rule, are quite different. This anomalous result may be due to sampling error, to some peculiar meteorological or other environmental conditions in 1959 or may indicate a real difference between this colony of the species and those at Wombat, Murrumbateman, Hall, Royalla "A" and "B" and Williamsdale. We would prefer not to speculate on this point until the Wallendbeen colony has been studied in a subsequent year.

The mean wet weight of each genotype, in the four colonies studied, with their corresponding variances, are given in table 1. It will be observed that the Wombat insects are the smallest and the Murrumbateman ones the largest, those at Wallendbeen and Royalla being of intermediate size. Since the various genotypes are far from equally abundant, we have to contend with very serious sampling errors in the case of the rarer ones, while the wet weights of the frequent genotypes have been determined

TABLE 1. *Wet weight of adult male individuals with different karyotypes at four localities*

Wombat, N.S.W. 1958–1959*

	St/St	St/Bl	Bl/Bl	
N	23	246	729	
$\bar{X}$	34.28	33.18	32.75	St'/St'
s^2	7.97	10.96	11.06	
N	3	65	271	
$\bar{X}$	35.00	32.53	31.75	St'/Td
s^2		15.10	8.68	
N	0	4	12	
$\bar{X}$		32.63	29.25	Td/Td
s^2		29.23	7.20	

Wallendbeen, N.S.W. 1959

	St/St	St/Bl	Bl/Bl	
N	27	138	220	
$\bar{X}$	37.06	35.37	34.51	St'/St'
s^2	15.94	14.80	11.68	
N	6	39	57	
$\bar{X}$	34.58	35.74	33.56	St'/Td
s^2	17.64	20.09	12.66	
N	1	2	4	
$\bar{X}$	38.00	39.25	35.25	Td/Td
s^2			3.42	

* Numbers of individuals different from those given by Lewontin and White (1960) since a later sample has been added.

Royalla, A.C.T. 1958

	St/St	St/Bl	Bl/Bl	
N	22	95	75	
$\bar{X}$	36.57	35.90	35.91	St'/St'
s^2	10.01	12.30	11.66	
N	8	57	64	
$\bar{X}$	35.69	35.65	35.34	St'/Td
s^2	5.85	14.10	16.45	
N	0	6	6	
$\bar{X}$		37.00	36.25	Td/Td
s^2		15.10	19.88	

Murrumbateman, N.S.W. 1959

	St/St	St/Bl	Bl/Bl	
N	107	359	262	
$\bar{X}$	41.34	40.52	39.90	St'/St'
s^3	18.49	18.02	17.67	
N	34	89	84	
$\bar{X}$	39.91	39.03	37.56	St'/Td
s^2	12.40	17.04	19.89	
N	0	3	5	
$\bar{X}$		37.67	37.90	Td/Td
s^2			2.93	

N = Number of individuals, $\bar{X}$ = mean wet weight (mg), s^2 = estimated population variance.
St = Standard sequence of the CD chromosome.
St' = Standard sequence of the EF chromosome.
Bl = Blundell sequence of the CD chromosome.
Td = Tidbinbilla sequence of the EF chromosome.

with considerable accuracy. The sampling error is particularly serious in the case of the three genotypes in which the Tidbinbilla sequence is homozygous. Only at Wombat do we have enough Bl/Bl, Td/Td individuals to calculate the mean weight with reasonable accuracy.

Certain regularities are immediately apparent from an inspection of table 1. In all four samples the St/St, St'/St' genotype appears to be the heaviest. At Wombat the Bl/Bl, Td/Td individuals seem to be the lightest. In the other three samples the Bl/Bl, St'/Td individuals seem to be the lightest. The scale of these differences may be appreciated from the fact that the St/St, St'/St' individuals appear to be from 3.5% to 17.2% heavier than the lightest genotype (Bl/Bl, Td/Td or Bl/Bl, St'/Td as the case may be). The other genotypes are intermediate and the data as a whole strongly suggest that, as far as wet weight is concerned, St/St >

St/Bl > Bl/Bl and St′/St′ > St′/Td. Whether St′/Td > Td/Td is more doubtful. At the Wombat locality this appears to be so, but in the other populations we seem to have St′/Td ⩽ Td/Td. The rarity of Td/Td individuals in natural populations renders this a difficult question to resolve. From a developmental standpoint it seems illogical that one Td chromosome should decrease the size of the insect and two Td chromosomes increase it once more (i.e. St′/St′ > St′/Td < Td/Td), but such may actually be the case. Far more probably, in view of the small number of Td/Td individuals in our material, the explanation of the discrepancy lies simply in sampling error.

Statistical Analysis

Tests of significance, to determine whether the apparent effects of the inversions on the weight of the insects are genuine, present certain difficulties. In the Royalla and Wallendbeen samples the variances of the various genotypes are not significantly different but in the considerably larger samples from Wombat and Murrumbateman heterogeneity of variances, significant at the 5% level, is revealed by Bartlett's test or by variance ratio tests for pairs of genotypes. Analysis of variance is hence contra-indicated, as a technique for those populations. We have accordingly resorted to comparisons between pairs of genotypes, neglecting the St/St, Td/Td and St/Bl, Td/Td ones, of which too few individuals were available. For the remaining 7 genotypes there are 21 pairwise comparisons, or 84 altogether, for the four samples. Of these 84 comparisons nine show significant heterogeneity of variances, at the 5% level. It might have been more satisfactory to apply the Aspin-Welch test (Pearson and Hartley 1956, table 11), rather than the t-test, in the case of these nine comparisons. But since tables for the Aspin-Welch test are only available for the 1% and 5% levels of significance, we have preferred to use t-tests throughout.

In table 2 we give the P values for the 21 comparisons, in the case of each of the four populations. It must be pointed out that 10 of the 21 comparisons are ones involving small numbers of one or both genotypes, and that some inconsistency is to be expected in the case of these. Moreover, two of the comparisons are between genotypes (St/Bl, St′/St′ and St/St, St′/Td on the one hand and Bl/Bl, St′/St′ and St/Bl, St′/Td on the other) where our genetic hypothesis does not allow us to predict which should be the heavier—in fact if the two chromosome pairs were exactly equal in the magnitude of their effects, these pairs of genotypes should show no difference in weight.

It will be seen that none of the differences between means are significant at Royalla, while in all the other three samples some of the differences are very highly significant. We might conclude from this that the inversions are having a significant effect on weight at three localities, but not at Royalla. However, since the differences between means are, in general, in the same direction at Royalla as in the other three localities, it seems more probable that the effect is simply weaker at Royalla, i.e. too weak to be demonstrated in a sample of 333 individuals.

In an attempt to combine the levels of significance over the four samples, we have in table 2 taken the normal deviates corresponding to the P values, summing them and dividing by $\sqrt{N}$ (i.e. by 2 in this case) to obtain P values for the 21 pairwise comparisons in the data as a whole. It will be observed that precisely in the two cases where we do not expect differences between the means the P values are highest; while in all those comparisons where we do expect differences and where the numbers of individuals are adequate, highly significant values of P are obtained. The relatively few instances

TABLE 2. *Differences between means—P values and normal deviates*

Genotypes*	Localities				$\Sigma(x)/\sqrt{N}$	P
	Royalla	Wallendbeen	Murrumbateman	Wombat		
SSSS & SBSS	.4166	.0392	.0729	.1211		
	.8126	2.0654	1.7934	1.5500	3.11	.0019
SSSS & BBSS	.4145	.0004	.0027	.1764		
	.8162	3.5778	2.9952	1.3518	4.37	.00002
SSSS & SSST†	.4818	.2072	.0786	(.6983)		
	.7004	1.2619	1.7587	−.3877	1.67	.095
SSSS & SBST	.3136	.2268	.0001	.0523§		
	1.0078	1.2089	3.8063	1.9407	3.98	.00006
SSSS & BBST	.1995	.0001	<.00001	.0001		
	1.2834	3.8000	5.9440	3.9652	7.50	<.00001
SSSS & BBTT†	.8430	.3858	.0783§	<.00001		
	.1981	.8676	1.7611	5.2952	4.06	.00005
SBSS & BBSS	(.9630)	.0274	.0649	.0811		
	−.0465	2.2058	1.8462	1.7444	2.87	.0041
SBSS & SSST†‡	.8674	.6235	.4195	(.3446)		
	.1670	.4909	.8073	−.9451	0.26	.795
SBSS & SBST	.6776	(.6184)	.0025	.0006		
	.4158	−.4981	3.0300	3.4320	3.19	.0014
SBSS & BBST	.1348	.0731	<.00001	<.00001§		
	1.4953	1.7921	5.8023	5.1725	7.13	<.00001
SBSS & BBTT†	(.8160)	.6285	.1691§	.00005		
	−.2327	.4838	1.3757	4.0403	2.83	.0047
BBSS & SSST†	(.8562)	.9609	.9924	.0002		
	−.1813	.0490	.0095	3.6994	1.79	.0735
BBSS & SBST‡	.6734	(.0489)	.0895	.6159		
	.4217	−1.9695	1.6979	.5017	0.33	.741
BBSS & BBST	.3622	.0631	<.00001	.00004§		
	.9114	1.8588	4.3850	4.3415	5.75	<.00001
BBSS & BBTT†	(.8210)	(.6679)	.2877§	.0003		
	−.2263	−.4291	1.0634	3.6261	2.02	.0435
SSST & SBST†	.9778	(.5572)	.5244	.2883		
	.0279	−.5872	.6367	1.0122	0.54	.589
SSST & BBST†	.3748	.5126	.0070	.0587		
	.8876	.6548	2.6966	1.8901	3.06	.0022
SSST & BBTT†	(.7657)	(.7761)	.2226	.0095		
	−.2981	−.2845	1.2198	2.5935	1.62	.105
SBST & BBST	.6613	.0097	.0242	.0737§		
	.4382	2.5877	2.2565	1.7889	3.54	.0004
SBST & BBTT†	(.7151)	.8345	.5448§	.0069		
	−.3651	.2090	.6058	2.7011	1.58	.114
BBST & BBTT†	(.6023)	(.3537)	(.8402)§	.0039		
	−.5213	−.9295	−.2016	2.8890	0.62	.535

* In abbreviated form, e.g. SBST = St/Bl, St′/Td.

† Comparisons involving small numbers of individuals (<10) in the case of one or both genotypes.

‡ Comparisons where we have no genetical reason for expecting a difference in either direction.

Figures in parentheses represent the P-values of differences between means which are in the opposite direction to that expected on the hypothesis, or (in two cases‡) in the opposite direction to the other three samples.

§ Comparisons where significant heterogeneity between variances (at the 5% level) exists.

where the differences between means are in the opposite direction to that expected are insufficient to cast any serious doubt on the hypothesis.

Since we have performed a relatively large number (84) of t-tests on our data, the significance levels have been somewhat disturbed. In view of the very low and very high P values in table 2, we do not consider this a serious objection to the method employed.

Discussion

The statistical analysis confirms that the two Standard sequences are both weight-increasing chromosomes, while the Blundell and Tidbinbilla elements are size-decreasing. This is certainly so in three of the populations and probably also in the fourth. Biologically this seems to be a most remarkable result. It is perhaps not unexpected that size-increasing and size-decreasing genes should have accumulated differentially on alternative cytological sequences, although we are not aware that this kind of effect has been demonstrated for any other species. The really interesting thing is, however, that these effects seem to be consistent in four different geographically remote populations. The Wombat and Wallendbeen populations belong to the 17-chromosome race of *Moraba scurra*, the Murrumbateman and Royalla colonies belonging to the 15-chromosome race. The evolutionary origin of the 17-chromosome race probably goes back at least half a million and probably more than a million generations (i.e. years, since there is only one generation a year). No doubt some interchange of genetic material has occurred across the narrow zone of overlap between the two races (White and Chinnick, 1957). But in view of the very low vagility of this wingless insect, the fact that in the populations we have studied the cytological sequences have the same effects on size implies an amazing evolutionary stability for the size-determining gene complexes associated with the inversion sequences. These co-adapted blocks of genetic material have remained constant, as far as size-influencing genes are concerned, over a period of hundreds of thousands and perhaps millions of years!

The relationship between mean weight and relative viability in these cytologically polymorphic populations is an interesting one. Genotypes giving large phenotypes (St/St, St′/St′ and St/Bl, St′/St′) and small ones (Bl/Bl, St′/Td) have higher viabilities than ones producing intermediate sized phenotypes (Bl/Bl, St′/St′ and St/Bl, St′/Td). But the genetic equilibrium in these populations is such that the commonest genotype (Bl/Bl, St′/St′) is one producing intermediate size (except at Murrumbateman and Royalla, where the commonest genotype is St/Bl, St′/St′). The two ends of the "adaptive ridges" in the topographies of Lewontin and White (1960) correspond to large and small phenotypes, respectively; but the natural populations occupy an intermediate part of the ridge (the "saddle").

This relationship may be regarded as a result of a special type of disruptive selection (Mather, 1955; Thoday, 1958). It fits in well with the idea that genotypes at opposite ends of the adaptive ridge are, to some extent, ecologically specialized to occupy different niches in the general environment so that, as a consequence, their selective values are frequency dependent. If genetically large individuals occupy one kind of niche and genetically small ones a different type of niche, any increase in the frequency of a particular genotype will lead to a situation in which it "spills over" into micro-environments to which it is less well adapted and in which its selective value is less.

It will be observed that this interpretation is closer to the viewpoint of Dobzhansky (daCunha, Dobzhansky, *et al.* 1959), according to which the different cytological genotypes in a panmictic popu-

lation are "ecological specialists," than to that of Carson (1959) who favors a simpler hypothesis of "pure heterosis" as the explanation of genetic equilibrium. But although we believe Dobzhansky's interpretation agrees better with the observed facts in *Moraba scurra* as far as the "adaptive ridge" is concerned, there is no particular reason to believe that it applies along the axis perpendicular to the ridge. The viability of St/St, Td/Td individuals is probably so low that this genotype cannot be regarded as adapted to any environmental niche; but the abundant Bl/Bl, St'/St' genotype may be ecologically unspecialized as well as intermediate in size.

There are two types of possible explanation of the effect of the cytological sequences of *Moraba scurra* on size. According to the first, the inversion sequences act directly on certain developmental patterns leading to large or small phenotypes. According to the second type of explanation the karyotypes cause immature individuals carrying them to occupy certain specialized ecological niches which determine whether they will grow into large or into small adults. We have no evidence as to which type of interpretation is correct.

In the case of the CD chromosome, there seems to be little or no indication of any dominance in the determination of wet weight, the heterozygotes being intermediate between the two homozygous genotypes and about mid-way between them. The situation in the EF chromosome might be interpreted as evidence for dominance of the size-reducing genes in the Tidbinbilla chromosome. But we really know far too little about the mean weights of Td/Td individuals to draw such a conclusion.

Since some heterogeneity of variances was detected in the larger samples (Wombat and Murrumbateman), we may ask whether this is a consistent and biologically significant phenomenon. The evidence on this point is contradictory. At Wombat and Wallendbeen there is a suggestion that the double heterozygotes have higher variances. But this trend is not shown by the samples from Royalla and Murrumbateman. It can thus hardly be regarded as signficant in spite of the fact that it was shown by the Wombat population in both 1958 and 1959. In the Wombat, Wallendbeen and Murrumbateman samples, in spite of small numbers, there seems to be a tendency for the Bl/Bl, Td/Td individuals to have lower variances than the other genotypes. But this trend is contradicted by the Royalla sample, where the six individuals of this genotype have the highest variance of all.

The populations we have studied represent a particular type of population, which has been found at many localities on the southern tableland of New South Wales. In these populations the frequency of Blundell always exceeds that of the Standard CD, while the Tidbinbilla sequence has a frequency of 10–35%. But other types of populations exist in this species, in some of which Standard is more frequent than Blundell, while in others a third CD sequence, Molonglo exists instead of or in addition to the Standard one. And near Berridale, N.S.W., yet another CD sequence, which we have called Snowy, exists together with Blundell, Standard and Molonglo being absent from that locality. It is, of course, by no means certain, or even likely, that the effects of the sequences we have studied on the wet weight of the insects are precisely the same throughout the whole distribution area of the species.

There is some reason to believe that the "Standard" sequences of the CD and EF chromosomes (which are both metacentric) represent the ancestral ones and that the Blundell, Molonglo and Tidbinbilla sequences (which are acrocentric) were later evolutionary derivatives, by pericentric inversion. The reasons for making these assumptions are different for the CD and EF pairs. As far as the CD pair is concerned, all the species most closely related to *M. scurra*, and indeed

TABLE 3. *Overall mean weights of the four populations*

Locality	Overall mean weight (mg)	Frequency standard CD	Frequency standard EF
Wombat	32.59	.14	.86
Wallendbeen	34.91	.25	.88
Royalla	35.82	.33	.77
Murrumbateman	39.99	.39	.88

the great majority of species of Morabinae, have a metacentric CD element with somewhat unequal limbs, i.e. closely resembling the Standard CD of *M. scurra*. Acrocentric CD's are only known in very few species, where they have clearly arisen by pericentric inversion. As far as the EF is concerned, this is represented in related species by separate acrocentric "E" and "F" chromosomes which must have fused to form a metacentric EF (i.e. one of the Standard type) at an early stage in the evolutionary history of *M. scurra*. The acrocentric Tidbinbilla type must hence have arisen later.

If the above interpretation is accepted, one might speculate that originally *M. scurra* was a slightly larger insect than in most present-day populations, and that the Blundell and Tidbinbilla sequences, with their size-reducing properties, enabled it to invade or exploit new ecological niches. Such an interpretation, although mere guesswork, may be tested to some extent, by comparing the mean size of the insects in many different localities, including some at which it is cytologically monomorphic.

We might perhaps expect to find a correlation between the mean size of all the individuals in a colony or deme and the frequencies of the size-increasing and size-decreasing chromosomes (i.e. one might predict that populations of small individuals would have high frequencies of the Blundell and Tidbinbilla sequences, while colonies in which the mean size is large would have high frequencies of the two Standard sequences). This correlation apparently exists in the case of the CD chromosome, but is not evident in that of the EF element (table 3).

SUMMARY

In four cytologically polymorphic populations of the Australian grasshopper *Moraba scurra* the "Standard" sequences of two chromosome pairs are weight-increasing, while the alternative "Blundell" and "Tidbinbilla" sequences are weight-decreasing.

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GENETIC VARIABILITY IN KOREAN POPULATIONS OF *DROSOPHILA MELANOGASTER*

YONG KYUN PAIK

Department of Biology, National Chunnam University, Kwangju, Chunnam, Korea

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Concealed genetic variability has been found by many workers in various species of Drosophila. Much of this work is reviewed in Spencer (1947). Notable among other studies are those of Chino (1936a, 1936b and 1937) on Japanese populations of *D. virilis* and of Stone and his group (1957) on irradiated Pacific island populations of *D. anannasae*.

In general it is clear that natural populations of many Drosophila species carry large amounts of heterozygus genetic variability, with marked quantitative and qualitative differences, however, between both intra- and inter-specific populations. In addition, rates of change in genetic variability within populations have sometimes been reported as rapid (Dubinin, 1946), or slow (Ives, 1945). A primary step in speciation and organic evolution is the occurrence, dissemination and frequency distribution of mutations in natural populations. The quality and quantity of mutations which accumulates in space and time within a population or species plays a significant role in the evolutionary changes occurring in the species.

Little is actually known about the mechanisms which control these factors in natural populations, although some progress has been made in elucidating concepts bearing on these mechanisms. Of particular use in this respect have been lethal mutations, whose detection is relatively simple and easy. The frequency of such mutants in a population is related at least theoretically to the breeding structure of the population and to the nature of its environment.

If there are no heterozygous effects, the concentration of recessive lethals at equilibrium should be lower in small populations than in continuously large populations (Wright, Dobzhansky and Hovanitz, 1942; Dubinin, 1946). Further, their frequency should be lower in small populations, at comparable generations, even before equilibrium is reached (Prout, 1954). Lessening of inbreeding at any time in a population leads to an increase in frequency of recessive lethal, or partially lethal, genes in the population's gene pool. Hence the frequency of these mutations found in a sample of flies from a population is in a sense a "yardstick" for estimating comparative sizes of populations (Ives, 1945) excepting as a selection operates appreciably for (Mazing, quoted in Dubinin, 1946) or against (Wright *et al.*, 1942; Stern *et al.*, 1952; Cordeiro, 1952; Prout, 1952) such heterozygous mutations.

There is increasing evidence of geographical differences in concentration of lethals and other genetic variability. Dubinin (1946) also reported periodical fluctuations in lethal frequency correlated with seasonal oscillations in population size, a phenomenon not confirmed by others (Wright *et al.*, 1942; Ives, 1954; Goldschmidt *et al.*, 1955). Further work is needed along this line.

In a large breeding population gene frequencies are determined by counteracting, systematic evolutionary forces—mutation, selection and migration. In a small, island-type population, even in one subject to oscillations in climatic and ecological conditions, random changes in gene frequencies are also important, for they will sometimes by themselves cause fixation or elimination of certain alleles without respect to their selective value

(Spencer, 1947). This aspect of population dynamics also needs more study at the level of natural populations.

The present study has been undertaken chiefly to determine the incidence of genetically deleterious second chromosomes in three Korean populations of *D. melanogaster* and to attempt to provide answers to the following guestions.

1. What is the frequency of lethal and other deleterious genes in these populations?
2. What can be said of population structure and size in Korea?
3. What is the genetic nature of the lethal choromosomes?
4. Are lethal genes distributed at random in the second chromosome of these populations?
5. Are the genetic structure and evolutionary trend of Korean populations of this species comparable to those of populations in other geographic areas?

A preliminary report of part of this work was presented by the author in DIS-32 (1958).

Distribution of D. melanogaster in Korea

Drosophila melanogaster, an originally tropical species, appears now to be cosmopolitan in distribution (Patterson and Stone, 1952). From collection records in Korea (Kikkawa and Peng, 1938; Paik and Kim, 1957) this species appears to be distributed throughout this country, always in association with human habitation or with such human activities as fruit orchards and vinegar or alcohol manufacture, often breeding in the small vinegar containers of private homes. In general, populations are confined to small domestic areas. In the semi-wild condition of large fruit orchards the species usually forms sympatric populations with a closely related species, *D. auraria*, which generally predominates. Fruit itself is available in the orchards only from mid-July through mid-September at which time orchard populations of *D. melanogaster* reach peak size.

Outdoor populations decrease gradually in size from mid-September until sharply reduced by frost usually late in October. Daily minimum temperatures which are generally below freezing prevent outdoor breeding of the species from December through March. The climate is characterized by average daily mean temperatures under 10° C from November through April and above 20° C for only about three months of the year, with a peak level normally in August or July.

Description of Collections

Three widely separated populations of *D. melanogaster* have been studied, in Taegoo (35.52° N, 128.35° E), Najoo (35.09° N, 126.55° E) and Quilpart (33.20° N, 126.32° E).

The first population sample was taken in mid-September 1957 near a fruit storage house in a large orchard district in rural Najoo. Five trap cans, 28 × 26 cm, containing fermenting apple bait, were set up there for two days after which about 500 flies were collected by sweeping with a net over the cans during a three hour period. A second collection was taken similarly late in August 1958. These samples will be referred to as NJ-57i and NJ-58h respectively.

Another sample was collected in a large brewing plant in Taegoo, one of the largest fruit farming districts in Korea, in mid-October 1957, at a time when no flies came to traps placed in the orchards. About 400 flies were collected by sweeping over rice fermentation tanks during a two day period. This sample is designated TG-57j.

The final sample was from an orchard area on the island of Quilpart on the southeastern tip of Korea. Here, too, flies failed to come to traps placed for several days in the orchards. About 400 flies were collected by sweeping over thirteen trap cans of yeasted apple and

TABLE 1. *Classification of chromosomes on the basis of F_3 viability*

Collection	Chrom's	le	%	sle	%	sv	%	Norm	%	Excep.	%
NJ-57i	171	18	10.5	5	2.9	5	2.9	143	83.7	0	0.0
NJ-58h	139	9	6.5	2	1.4	8	5.8	120	86.3	0	0.0
QI-57i	147	11	7.5	0	0.0	8	5.4	128	87.1	0	0.0
TG-57j	154	15	9.7	8	5.2	6	3.9	101	65.6	24	15.6
Total	611	53	8.7	15	2.5	27	4.4				

grape mash placed among twenty-five large molasses fermentation tanks in an alcohol plant during a five day period. This sample is designated as QI-57i.

METHODS OF ANALYSIS

Genetic analysis of the collected flies involved the use of *Bl* L^2/Cy, sp^2 following a scheme similar to that used by Ives (1945) and others, the purpose being to test the homozygous effects of one choromosome from each collected fly used in these tests. Collected males were used from the NJ and TG samples. In the QI tests third generation male offspring from collected females were used, each female line having been maintained as a separate stock.

In the test generation matings, seven to ten pairs of Cy F_2 were mated for each test line, using bottle cultures. These parents were transferred at three to four day intervals to one or two additional cultures, all of which were kept at $25 \pm 1°$ C. Culture medium consisted of a standard corn meal-molasses-agar mixture containing 0.5% propionic acid as a mold inhibitor and innoculated with Wagner's Y-2 yeast strain.

The F_3 flies were counted at three or four day intervals through the tenth to twelfth day after emergence of the first flies. Total counts numbered at least 500 flies in most lines, except for some cases in TG-57j where counts ranged from 60 to 200 flies.

PRESENTATION OF DATA

The results of the F_3 analysis are presented in table 1, in which 611 chromosomes are separated on the basis of their proportion of non-Cy flies into five classes: Lethals (le)—less than 3.34% non-Cy F_3 flies, semi-lethal (sle)—3.34 to 16.66% non-Cy F_3, subvital (sv)—16.67 to 26.67% non-Cy F_3, normal (norm)—26.67 to 39.99% non-Cy F_3, and exceptional (excep.)—more than 39.99% non-Cy F_3 flies. Total counts in the tests were large enough (average of 700 flies per line) so that only seven lines are not significantly different (at the 95% confidence level) from the arbitrary limits of their classes. It may be concluded therefore that the chromosomes are accurately placed within these classes.

In each of the four population samples represented in table 1, the normal class is by far the largest group. The percentages of normal in the QI and two NJ samples are statistically similar ($P = 0.79$ by chi square). The percentage of normal in the TG sample is lower than the average of the other three ($P = <.001$) but is brought up to their level ($P = 0.29$) if the 24 exceptional cases are included in this group.

The proportions of lethal, semi-lethal and subvital chromosomes are low in all four samples, probably too low to give an accurate estimate of the true proportions of these types in the natural populations. The actual distribution of lethal and sub-vital chromosomes in the four samples appears to be random ($P = 0.58$ for each distribution), while that of the semi-lethal cases shows more skewness but with too low a total number to make the skewness of practical significance.

TABLE 2. *Per cent non-Cy F_3 in exceptional TG-57j lines*

Line	Cultures	Total F_3	% non-Cy	Line	Cultures	Total F_3	% non-Cy
99	3	261	45	156	3	205	60
129	4	712	46	142	3	262	65
244	1	389	46	91	2	226	68
152	3	801	47	107	2	441	67
4	2	491	48	b126	4	307	67
b189	3	62	52	203	2	220	67
b220	2	706	53	121	3	266	73
b157	3	166	57	92	4	1,042	73
140	3	241	52	112	2	100	85
168	2	371	53	177	2	162	90
247	2	155	54	s244	1	125	93
124	3	179	58	200	1	123	92

The distribution of the combined group of lethal and semi-lethal chromosomes is not as close to random as that of the lethals alone but the deviation is still not statistically significant ($P = 0.087$). The two NJ samples do not differ significantly from each other in any category, and show a P value of 0.11 for combined frequencies of lethal and semi-lethal chromosomes.

The 24 exceptional chromosomes of the TG-57j sample represent cases where the proportion of non-Cy F_3 was far in excess of the expected 33.3%. They ranged, in fact, from 45% to 93% non-Cy F_3 in counts which ranged from 62 to 1042 flies per line. The data for the F_3 counts of these lines are given in table 2. It seems doubtful to the author that modification of the selective viability of the non-Cy flies could in itself produce so striking a deviation from expectation. It seems more likely that some factor such as "segregation-distortion" (Sandler *et al.*, 1959) is the cause of the appearance of too many non-Cy and too few Cy F_3 flies in these lines, particularly in those lines with more than 50% non-Cy flies.

TABLE 3. *Percent non-Cy in retest matings of some TG-57j exceptional lines*

Line	Total progeny	% non-Cy
99	356	23
b189	599	29
168	583	33
220	545	37
157	203	36
124	541	35
156	489	33
142	647	34
107	625	29
203	577	37
92	259	34
121	381	36
s244	436	32

A portion of the exceptional lines was retained, carried each generation by selecting Cy parents, and tested again thirteen generations later. The results from the retest counts are given in table 3. They show no similarity to the original F_3 counts given in table 2. All lines in table 3 show ratios falling within the normal percentage class of table 1, excepting line 99 which appears now to have become a sub-vital chromosome. It would be expected, from the nature of known segregation-distortion factors, that any such factor present in the lines studied here would have been lost during the thirteen generations of stringent selection against its effects.

One additional point of interest about line 99 is the possibility that in the first counts, in table 2, it represents the combined effect of segregation-distortion and a gene with less than normal homozygous viability.

During the classification of the F_3 flies in the foregoing tests of the 1957 collections visible mutations were also looked

TABLE 4. *Frequency of visible mutants associated with the sub-vital and normal chromosomes of the 1957 collections*

Collection	Chromosomes	Visibles (+)	Visibles (−)	Total %	Frequency of appearance 1	2	3	4	5
NJ-57i	148	4	4	5.4	1	2	1	0	0
TG-57j	131	7	3	7.6	3	1	0	0	1
QI-57i	136	6	4	7.4	4	3	0	0	0
Total	415	17	11	6.8					

(+) and (−) denote complete and incomplete penetrance, respectively.

for in the non-Cy flies. The number of non-Cy appearing in semi-lethal lines is too low to make classification of visibles in those cases as reliable as in the sub-vital and normal lines. Accordingly visible mutation frequencies have been based only on the latter two viability classes, excluding the few cases of strong visible mutants associated with semi-lethal chromosomes. The frequencies of visibles in the three population samples is shown in table 4.

As indicated in the table more than half of the visibles occurred more than once within their respective populations, but the total incidence of visibles was low in each population. No localization or allelism tests were carried out with these visibles. On the basis of their phenotypic similarities, the following loci described in Bridges and Brehme (1944) appear to be represented: in NJ—*c*, *cn* and *nw*^{2}; in TG—*b*, *mr* and *stw*; and, in QI—*ab*, *ae*, *al*, *ap* and *b*. There were found in addition one visible in NJ, two in TG and two in QI which do not correspond to any loci described in Bridges and Brehme.

The mutant *b* was the only one found in two different areas. It occurred five times in TG and only once in QI. In TG it was associated four times with exceptional lines represented in tables 2 and 3. Its multiple appearance in TG can be considered therefore either a result of excessive inbreeding in the population or a result of an association with the segregation-distorter genetic factor which it is assumed caused the appearance of the exceptional chromosomes. Indeed the joint occurrence of *b* and distortion of the Cy : non-Cy ratio is additional suggestive evidence of the location of a genetic factor for segregation-distortion in these chromosomes and in the population at Taegoo.

The low frequency of lethal and semi-lethal chromosomes and the high proportion of duplicate visible mutants so far reported here both suggest that the breeding population unit in these population areas is small. Further and more direct evidence along this line should be obtained by determining the amount of allelism among the lethal chromosomes. If the breeding population unit is truly small then the amount of allelism among the lethal chromosomes within each population sample should be much greater than the amount in cross tests of lethals from different samples.

All of the available strictly lethal chromosomes (no non-Cy F_3) of the different samples have been used to test the above point. Cross tests were raised in bottle cultures using seven males of one line times seven females of another. The cultures were raised at 25° C and were counted in the same manner as in the original F_3 tests. A given pair of lethals was considered to be alleles if the number of non-Cy offspring amounted to less than 3.34% of a total count of not less than 60 flies. The results of these tests are given in tables 5 and 6.

A comparison of the rates of allelism

TABLE 5. *Frequencies of alleles and of allelic lethals within populations*

Sample	Lethals	Crosstests	Allelic crosses	Allelism rate (%)	Frequency of Appearance					
					1	2	3	4	5	6
NJ-57i	18	153	12	7.8 ± 2.2	5	3	1	1	0	0
NJ-58h	9	36	15	41.2 ± 8.2	3	0	0	0	0	1
TG-57j	15	105	16	15.2 ± 3.5	7	1	0	0	0	1
QI-57i	11	55	1	1.8 ± 1.8	9	1	0	0	0	0

Heterogeneity between samples

	d/SE_d	P
NJ-57i vs. NJ-58h	5.2	.0001
NJ-57i vs. TG-57j	1.9	.06
NJ-58h vs. TG-57j	3.4	.0006

in the intra-population tests with those in the inter-population tests shows at once that the former are indeed much larger than the latter. The additional data on the frequency of appearance of the different lethals within each population sample shows that most of the allelism arises from the multiple occurrences of only a few of the lethals, probably as a result of genetic drift. These observations verify the interpretation based on frequency of lethals and on the data from visible mutants, that these Korean populations are small breeding units. It is abundantly clear that inbreeding serves to keep the lethal frequency low in these areas.

The data of table 5 also show a striking difference between the two Najoo collections in the amount of allelism observed, it being five to six times higher in 1958 than in 1957. The data from crosses between these two samples in table 6 suggest that there was practically a complete changeover of lethals in the population between seasons, and that the population must have decreased to a very small number during the intervening winter. In line with this interpretation is the fact that while in 1957 three of the lethals appeared, respectively, twice, three times and four times among the lethal chromosomes, in 1958 only one appeared more than once and it, a different lethal from those found in the 1957 collection, appeared six times. This accumulation of identical lethals could, however, be related to the segregation-distortion effects already considered.

TABLE 6. *Frequencies of allelic lethals in inter-population tests*

Populations tested	Crosstests	Allelic crosses	Allelism rate (%)
NJ-57i (18) × QI (11)*	198	0	0.00
NJ-57i (18) × TG (15)	170	0	0.00
TG (15) × QI (11)	165	1	0.61
Total	633	1	0.16

* Number in parentheses indicates the number of lethals used in the crosstests.

The comparatively low rate of allelism in the QI data of table 5 is not necessarily indicative of a larger breeding unit in this area. Several of the lethal genes in this sample might have arisen as new mutations in the three generations of laboratory culture of these lines before the lethal chromosomes were extracted. This would substantially lower the allelism rate compared to the other samples.

With the exception of one NJ-57i lethal which was lost, the lethal genes reported here in the 1957 collections have been tested for locus on the second chromosome, first by use of the *Bl* L^2 marker, by which the lethals could be differentiated as being in the left or right arm or middle of the chromosome, and then by use of *Sp J*, and/or *Pfd Pin* (these markers are described in Bridges and

Brehme, 1944) to place the lethal genes more accurately within each arm. These tests also revealed cases in which the lethal chromosome carried more than one lethal gene.

The distribution of lethal genes within the lethal chromosomes of the several samples is shown in table 7, in each case first in the chromosomes which had only one lethal gene per chromosome and then in the chromosomes which had two lethal genes per chromosome. In the former class all 28 lethals were in the middle or right sections of the chromosome. In the latter, 13 of the 26 genes were in the left arm. This suggests a non-random distribution of lethal genes within the population.

The distribution of non-allelic lethal loci represented in the tested lethal chromosomes is shown in table 8. It can be seen that the genes which contribute lethal mutations to these populations are apparently randomly distributed along the second chromosome.

The crossover tests revealed only three cases of the sharply reduced crossing over which is characteristic of chromosome aberrations in this species. All were in the NJ-57i sample. Two of these, showing only rare crossovers between *Sp* and *J* (which could have been cases of lack of penetrance of one of the markers) and no L^2 or wild-type flies in counts of 1,237 and 2,042 flies, respectively, in the *Bl* L^2 tests, appear to be left arm inversions. The third case showed comparatively poor fecundity in each of the three types of crossover tests. It showed no crossingover in 440 flies from the *Sp J* test but produced from four to six per cent of L^2 and wild-type and of *Pin* and wild-type in somewhat larger counts from the other two tests. The author interprets this case as probably a small deficiency within the *Sp J* area of the chromosome, but the possibility of a small inversion is not excluded. None of these cases was studied cytologically.

The two lethal chromosomes from TG-57j and QI-57i which proved allelic in the genetic cross test gave also a similar and interesting result in the crossover tests. The locus of the lethal in each case, on the basis of the *Bl* L^2 test, appeared to be at about 70, which is also the approximate locus of *Pfd*. When an attempt was made to secure *Pfd Pin*/lethal flies for a further check on the locus, no such flies appeared in several trials. This suggests that this particular lethal is allelic to *Pfd*, sharing with it the characteristic of lethal when homozygous but not containing the heterozygous phenotypic effect.

TABLE 7. *Distribution of lethal genes in the lethal chromosomes*

Collection	le genes/chrom.	Cases	Distribution		
			Left	Middle	Right
NJ-57i	1	12	0	6	6
	2	3	6	0	0
TG-57j	1	10	0	8	2
	2	5	1	3	6
QI-57i	1	6	0	1	5
	2	5	6	2	2
Total	1	28	0	15	13
	2	13	13	5	8

DISCUSSION

The foregoing data show that the concentration of recessive lethal second chromosomes in natural populations of Korean *D. melanogaster* is low, ranging from 6.5 to 10.5% in the four samples

TABLE 8. *Distribution of non-allelic lethal loci in chromosome 2*

Collection	Non-allelic loci	Left	Middle	Right
NJ-57i	11	6	3	2
TG-57j	14	1	6	7
QI-57i	15	6	3	6
Total	*39	13	12	*14

* Corrected for inter-allelic pair in TG × QI tests.

which were analyzed. The combined incidence of lethal and semi-lethal chromosomes ranges from 7.5 to 14.9%. The variations between areas and between two samples taken a year apart in one area are not statistically significant. The four samples were taken when the populations were near peak size.

These frequencies are generally lower than those reported by Dubinin (1946) who found frequencies of 20 to 25% in most Russian areas, with Gelendzhik, however, much lower and at a level comparable to that characteristic of these Korean populations. The frequencies reported by Ives (1945, 1954) for widely separated populations of North American *D. melanogaster* show a much higher range, from 28 to 65% of lethal and semi-lethal chromosomes. Goldschmidt *et al.* (1955) found 39% of such chromosomes in Israeli populations.

The second chromosome of *D. prosaltans* and *D. willistoni* corresponds to the second chromosome of *D. melanogaster*. In tests of Brazilian populations of these species, Pavan *et al.* (1951) found an average of 41% of lethal and semi-lethal chromosomes in *D. willistoni* and Dobzhansky and Spassky (1954) reported 33% in *D. prosaltans*. Marginal populations of *D. willistoni* in Brazil and in Florida, U.S.A., analyzed by Townsend (1952) and Cordeiro (quoted in Dobzhansky and Spassky, 1954), have yielded 28 to 31% of lethal and semi-lethal chromosomes. Wright *et al.* (1942), summarizing also earlier work of Dobzhansky, reported from 14 to 30% of lethals and semi-lethals in southern California, U.S.A., and in Mexican and Guatemalan populations of *D. pseudoobscura*, testing the third chromosome, which is homologous to only the right arm of the second chromosome of *D. melanogaster*.

From this brief review it appears that Korean populations of *D. melanogaster* have perhaps the lowest load of lethals yet reported for any species of Drosophila distributed over so wide an area. Taken in conjunction with the finding of a comparatively high frequency of allelism among the lethals which were extracted, ranging from 8 to 42% in the most critical samples, this indicates that the breeding population of this species in Korea is exceptionally small. The allelism data from inter-population tests also indicate that the populations in korea are sharply discontinuous in both space (geographical) and time (annual). The comparatively high frequency of certain lethals within each sample, and the fact that they differ from sample to sample, confirms the smallness of the breeding unit. There is nothing to suggest that a high rate of mutation at specific loci is the cause of this situation, but this possibility is not excluded. Of the other geographical areas thus far reported, only the Gelendzhik population analyzed by Dubinin in the 1930's presented a comparable picture, and it was not quite as extreme as these Korean populations.

Spencer (1946, 1947) from his studies on the frequency and distribution of visible mutations in North American populations of *D. immigrans* concluded that it breeds chiefly in small "island" populations in which genetic drift is pronounced due to periodical fluctuations in population size. He also concluded that the same situation exists in northern populations of *D. hydei* in the same continental area. Chino (1937) and his collaborators, studing Japanese *D. virilis* populations, have shown that it breeds in discrete populations characterized by population-specific visible mutants and much inbreeding.

In general, Korean populations of *D. melanogaster* seem to have a breeding structure more like these other species of Drosophila than like the populations of *D. melanogaster* studied by other workers. The low frequency of lethals, the high frequency of allelism among lethals, the presence of different duplicate

visibles in the areas studied, and the sharp discontinuity of populations both in time and space, all suggest that this is the case.

While the precise reason for this difference in Korean populations cannot be ascertained at present, the author has shown (Paik 1958) that populations of species of Drosophila decrease in size to very small numbers both in winter and in summer in one area of southern Korea. Considering also Wright's (1942) demonstration that effective breeding size (N) is much more closely related to the minimum size than to the maximum size of a population, one can suggest that climatic and ecological oscillations are the basic reason for the situation which exists in Korean *D. melanogaster* populations.

In one of the three areas, Taegoo, a peculiar class of chromosomes was found whose behavior was such as to suggest that this particular population carries a segregation-distorter factor similar in principle to one reported by Sandler *et al.* (1959) in a natural population of *D. melanogaster*. This suggests that the breeding structure of the Taegoo population may be more complex than that in the other two areas.

It is possible that frequent spraying of intense fungicides and insecticides in Korean orchards may be a factor in determining the genetic nature of their Drosophila populations. The spraying program in these orchards is one of two or three applications per month from April through mid-July. Ives (1954) suggested that such spraying might cause a decrease in size of orchard Drosophila populations. Ogaki and Tsukamoto (1953) and Tsukamoto and Ogaki (1953) found a few local strains of Japanese *D. melanogaster* which carry some degree of DDT resistance and showed that such resistance was not induced by the drug itself in laboratory tests. Kikkawa (1953) showed further that these strains are resistant also to seven other killing agents. The work of Tsukamoto, Ogaki and Kikkawa (1956) suggests that resistance, at least to DDT and BHC, is controlled chiefly by a dominant gene near locus 66 in the second chromosome.

The present study shows that the distribution of lethal genes in the two arms of the chromosome may not be random in natural populations of Korean *D. melanogaster,* particularly when single lethal chromosomes are considered. The total count for all chromosomes was 13 lethals in the left arm, 21 in the right arm and 20 in the middle region. Ives (1945), studying only non-allelic loci but working with a population where alleles were very infrequent, found a distribution of 49 in the left arm and 51 in the right. Twenty of his lethals were of laboratory origin. In the present study, non-allelic lethal loci were also randomly distributed: 13 in the left arm, 14 in the right and 12 in the middle. Wigan (in Hildreth, 1956) localized 17 "point" lethals in the second chromosome, finding 3 in the left arm, 9 in the right arm and 5 near the centromere. He found a similar distribution for another sample of 18 natural population lethals.

Taking all the information together the data suggest that the distribution of lethals in Korean populations resembles the findings of Wigan more than those of Ives. It is possible that this is another difference in genetic structure between Korean *D. melanogaster* populations and the Massachusetts, U.S.A. area studied by Ives. This situation may reflect a difference in mutability in the two areas.

Because of the small size of the breeding unit in Korean populations of *D. melanogaster* it is probable that its evolutionary development through the course of time in response to local ecological and bio-climatic conditions will be quite different than in other geographic areas where it apparently breeds in much larger units. Total amount of variability useful for genetic adaptation will be much less per breeding unit and the populations will

be more affected by genetic drift in Korean areas.

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Summary

1. Four samples of Korean *D. melanogaster* from three populations in fruit orchard areas were analyzed for viability factors and visible mutations in 611 second chromosomes by use of *Bl* L^2/Cy, sp^2. Lethals were tested for allelism and for chromosome locus. The combined frequency of lethal and semi-lethal chromosomes ranged from 7.5 to 14.9%, with no significant differences between areas or between collections taken a year apart in the same area. Allelism was comparatively very high in inter-population and low in intra-population tests and there appeared to be a complete turnover of lethals in the area sampled twice. Different recessive visibles characterized each area.

2. These findings suggest that *D. melanogaster* breeds in very small units in Korea, in contrast to its apparent population structure in other studied geographical areas and resembling instead certain populations of *D. hydei* and *D. immigrans* in U.S.A. and *D. virilis* in Japan.

3. The distribution of lethal genes may not be random in the chromosomes of Korean populations.

4. In one population, 15% of the chromosomes showed a large excess of non-Cy flies in the first test generation. This characteristic was completely absent when half of the lines were retested 13 generations later. It is suggested that a segregation-distortion factor may be present in this population.

5. Two lethals in one sample appeared to be associated with an inversion and a third with a deficiency.

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THERAPSIDS AS MAMMALS

LEIGH VAN VALEN [1]

Zoology Department, Columbia University, New York

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The therapsids are usually placed in the class Reptilia as its most mammal-like representatives. This is on the basis of the now commonly accepted criteria of jaw-ear structure, where mammals are defined as having (1) a dentary-squamosal suspensorium, (2) the articular-quadrate joint not a mandibular suspensorium, (3) three middle ear bones, and (4) the mandible containing only the dentary (Simpson 1959). It is the present thesis that (1) tetrapod classes should be defined on the basis of their major adaptive differences, (2) that the mammalian grade of adaptation was largely reached in the therapsids, and (3) that the therapsids should therefore be included in the Mammalia.

No specific mammal or reptile need be referred to; comparison will rather be with a construct similar to the *Typus* of the German idealistic morphologists, whose characters are observable in most species except for obvious specializations. The Theria as a whole and the Sauropsida (of Watson 1957) as a whole are the standards of comparison. If necessary, *Diadectes*, *Iguana*, *Didelphis*, and *Sus* may be kept in mind.

Much of the evidence for this view has been discussed by Brink (1957a); see also Broom (1932). The major adaptive distinctions of mammals as compared to typical reptiles will now be considered, together with any possible evidence that the therapsids had each of these characters in life. "The theoretical approach to any field of science is something of a luxury" (Burnet 1959). However, if the criteria of class placement are to be such as are largely unobservable directly in fossils, speculation one way or the other as to their condition in extinct groups is necessary.

The predominant adaptive features that distinguish the mammalian type as a whole from the reptilian seem to be increased amounts of the following: (1) care for the young, (2) intelligence (i.e. ability to learn), and (3) activity, perhaps the most important. There are exceptions in both directions, of course, but these are of interest largely because they are exceptional. With flight as a unifying factor, the same list seems to apply to birds. The great majority of mammalian characters can be associated with one or more of these somewhat related but largely distinct aspects.

REPRODUCTION AND INTELLIGENCE

With care for the young can be associated mammary glands, and perhaps separate anal and urogenital openings, neither of which is observable in fossils, and also vivipary and facial musculature (for suckling), which are potentially observable. Negative evidence here is of little significance because of the states of preservation and study required.

However, Brink (1955b) has recently described a primitive cynodont, apparently female, in the same nodule with which there was associated a tiny specimen of the same species, possible but far from certain inferences being of course vivipary or attention subsequent to hatching, with suckling neither excluded nor positively indicated. The probable development of homothermy and perhaps facial musculature in many therapsids, to be discussed below, is pertinent also. The weak teeth in the young of some cyno-

[1] National Science Foundation predoctoral Fellow, 1958–59; Columbia University Fellow, 1959–60.

donts are interpreted by Brink (1955a) as evidence for suckling, but it could equally indicate merely different food from the adult, whether milk, food (as insects) brought by the mother, or merely soft food sought by the young themselves. He also argues that vivipary is an adaptation to increase in skull size, but this seems tenuous (although possible) in view of diverse large-headed oviparous birds and reptiles.

Increased intelligence is associated in mammals with expansion of the cerebrum (especially the neopallium) and probably the improved regulation of the blood, including its temperature. Cerebral size is well known only in the cynodonts *Diademodon* (Watson 1913) and *Nythosaurus* (Simpson 1927), and inferred in the tritylodont *Oligokyphus* (Kühne 1956), in all of which it is still not much larger than in undoubted reptiles. Related to this, the frontals are still small and the squamosal is not part of the braincase.

Among Jurassic mammals, *Triconodon* has a slightly larger cerebrum, though of shape and relations similar to that of cynodonts (Simpson 1927), and a pantothere is probably similar. The cerebrum is larger in the Tertiary multituberculate *Ptilodus* (Simpson 1937), and considerably more so in the recent monotremes. In all these (except tachyglossids), however, as in the cynodonts, it is smooth or nearly so. Crompton (1955a, b) maintains that the brain could expand considerably only when the skull was no longer kinetic, as it apparently still was in at least an advanced therocephalian. An akinetic skull may be necessary, but it is certainly insufficient, as anomodonts had an at least mostly akinetic skull (Agnew 1959) but seem to have had a largely reptilian brain.

The olfactory lobes are known only in cynodonts, where they were remarkably large, probably in relation to a more active life. *Triconodon* is generally similar, but has them somewhat smaller; those of *Ptilodus* are very large, as in a Jurassic presumed multituberculate (Simpson 1927). The cerebellum is quite large in the cynodonts *Nythosaurus, Diademodon,* and *Thrinaxodon* (Olson 1944) and to some extent in their collateral descendent *Oligokyphus,* presumably related to muscular coordination, balance, and perhaps homothermy in a more active animal. In other therapsids in which the cerebellum is known, it is smaller but still usually larger than in typical reptiles (Olson 1944; Watson 1914, 1921). The pons, unknown in reptiles, is present in three of four anomodonts, a gorgonopsian, a therocephalian, and a cynodont (Olson 1944). This is perhaps also related to activity through its influence on breathing.

Activity

Activity is a broad and inclusive criterion. Locomotion, homothermy, digestion, and probably other categories can be treated as aspects of this. The only evidence available as to the presence or absence of a soft skin is that of the rhinarium, discussed below. Locomotor functions become for the most part mammalian in some therapsids: scapular spine and incipient supraspinous fossa, "rotation" of pelvis with a large obturator fenestra, forward expansion of the ilium, and backward expansion of the pubis, increase in number of sacral vertebrae, vertical position of the thinner limbs with a greater femoral trochanter and olecranon process, a calcanear tuber, and phalangeal reduction, are highlights of many similarities observed in the fossil record in various therapsids (e.g. Gregory and Camp 1918, Brink 1955a). Each of these can be related in detail to faster movement.

Differentiation of the now thecodont dentition into incisors, canines, and a reduced number of cheek teeth which often have cusps indicates greater efficiency in chewing food into smaller and more easily digestible pieces, with there-

fore a greater supply of potential energy assimilated in a given time. The several developments of a secondary palate are closely related to this, as are the development of a coronoid process on the dentary, movement of the masseter insertion from the angular to the dentary (Watson 1912), wider temporal fossae with the development of a mammalian zygomatic arch, and the controversial matter of the relation of the several intermediate types to the mammalian in the change from a reptilian unlimited and alternating tooth replacement to a mammalian single replacement (e.g. Kermack 1956, Crompton 1955c).

Mammalian homothermy directly involves, in addition to nervous control, the insulation of hair (or subcutaneous fat), the cooling by sweat glands (and mouth evaporation), a relatively high blood pressure with concomitant increased kidney tubule resorption (Smith 1953), and a higher blood temperature than in the usual immediate environment. Well-developed but unossified ethmoid turbinals, for cleaning, moistening, and warming air and for smell, also fit here and are known in *Diademodon* and *Nythosaurus* (Watson 1913). Turbinal processes from the septomaxillaries and mesethmoids are common. According to Kühne (1956), there are no ossified maxilloturbinals in *Oligokyphus*.

Evidence from the Snout

The best evidence now available for the presence of sweat and other skin glands, and perhaps facial musculature, a loose skin, and hair, is provided by various surficial features of the snout. Foramina for blood vessels and nerves, analogous to the mammalian infraorbital foramen, are present in the maxillary and sometimes also the premaxilla, lacrimal, nasal, and dentary of many and diverse therocephalians and gorgonopsians (Broom 1920, 1936a; Broom and Robinson 1948; Watson 1931; von Huene 1950; Brink 1954, 1959; Brink and Kitching 1953a; Crompton 1955a; Attridge 1956), and are the rule in cynodonts (e.g. Simpson 1933, Parrington 1934).

These vary from a couple of small openings, as in *Cistecynodon* (Brink and Kitching 1953b), to numerous ones that include a remarkably large foramen below the septomaxillary foramen, opening forward into a groove, in *Ericiolacerta* and a few other therocephalians.

Estes (in press) has pointed out that generally similar foramina are found in lizards and other reptiles, with one closely analogous to that of *Ericiolacerta* variably present in the teiid *Tupinambis*. These foramina, however, seem from their positions and relative development to be mostly related to the development of the teeth. The foramina in therapsids are perhaps of a similar nature, or they may have led to different structures in different genera. Usually there are some on the lateral surface of the maxilla, perhaps to supply the muscles of a movable lip. The large one mentioned above, however, may conceivably have been related to a great development of tactile sense organs anterior to it, with or without an accompanying movable and muscular extension of the snout, i.e. a rhinarium. "It is unlikely, though, as the evidence of the duck's bill shows, not incredible, that this development should have taken place in a skin covered with normal reptile-like scales" (Watson 1931). Broom (1903), describing a prominent longitudinal ridge low on the maxillary of a therocephalian, thought that a fleshy lip hung from this.

The musculature of the lip and adjoining area made credible by these arguments was conceivably related (or at least preadapted) to the development of milk glands, although it is probably more explicable, if present, as providing a means of obtaining small, softish food and keeping it in the mouth as it was chewed.

Direct evidence for hair has been claimed by Brink (1957a, b, not Watson, whom he cites) in *Ericiolacerta* and another therocephalian. This is the presence

of pits in the anterior part of the maxillary, which suggest to him the existence of vibrissae there. Although this is possible, similar pits in another therocephalian were interpreted by Broom (1920) as related to glands in the skin, and a horny or scaly covering is not really excluded, as pointed out by Broom (1936a) for still other instances. These last cases (of Broom 1936a) are interesting in that no nutrient foramina were detected, making doubtful the sensory interpretation at least here. *Cynognathus* itself has pitted and rugose bone in this region (Seeley 1895), the gorgonopsians *Lycaenops ornatus* (Broom 1930b) and *Rubidgea platyrhina* (Brink and Kitching 1953a) have different types of ornamentation on the snout, and other cases of questionable interpretation are also known. Some are probably analogous to the ornamentation of later dinocephalians. However, the presence of hair is made quite possible by the probable homothermy (below), and the presence of vibrissae (only occasionally leaving evidence in the bone) is by no means excluded.

A pit anterior to the orbit is present in many therocephalians (e.g. Boonstra 1934b, 1954), gorgonopsians (e.g. Boonstra 1934a), and cynodonts (Seeley 1895, Parrington 1946, Brink 1955a and 1957a). It is quite variable in size, depth, and, to some extent, in position, and is undoubtedly not everywhere strictly homologous. In fact exceptionally two pairs may be present. In the therocephalian *Euchambersia* (Broom 1930a, 1936b) it is much enlarged and runs into a canal leading towards the canine, which has a unique ridge on its side. Nopcsa (*fide* Boonstra 1934b) suggested that here it was for a poison gland; this seems more reasonable than any other proposal and has been accepted (e.g. by Watson and Romer 1956).

The function in other cases remains, however, less clear. In some specimens of *Diademodon* (Brink 1955a, 1957a) grooves arborize from it toward the external nares. Therefore the pit is here believed to house a gland for moistening the snout; Brink (1959) describes a quite different set of grooves below the nares as having a similar function in the therocephalian *Moschorhinus*. If this glandular nature be accepted, it provides further evidence of a rhinarium, but the question remains as to the origin of the glands.

Brink considers that they were derived from sweat glands, as similar epidermal glands are virtually absent in known reptiles. There is, however, an analogy in the poison gland of *Heloderma*, crocodilians have musk glands, and birds have developed a large uropygial gland. Their origin, if indeed the glands were present, must remain uncertain, although the very probable presence of sweat glands in the unquestioned mammals that are the not so very distant descendants of some of these forms might incline us somewhat to accept this origin here. And since milk glands are modified sweat glands, if the former were present in any therapsid the latter must have evolved considerably earlier.

Other Evidence of Mammal-like Adaptation

The presence of numerous non-reptilian characters in both the living monotremes and therians can be interpreted either as independent acquisitions some time after divergence within the reptilian grade or as characters present in their common ancestor somewhere within the therapsids. The greatly enlarged cerebrum, jaw-ear structure, and some other osteologically determinable features are quite probably in the former category. As for hair, milk glands, etc., there is no basis for judgment except such speculations as we can make about the therapsids. Although poor evidence by itself, the argument from the probable polyphyletic origin of traditional mammals (i.e. that some characters may fall in the second

class) is at least well in keeping with the conclusions from other lines of evidence. And the fact that the monotremes are oviparous does not imply that the therapsids ancestral to the therians also were, because these groups could have been separate throughout the Triassic. Olson (1944) in fact considers the cerebellum of one cynodont to have been more advanced than that of monotremes.

The division of the ventricle and reduction of aortic arches, used by Goodrich (1916, see also von Hofsten 1941) as a basis for dividing the Reptilia into Theropsida and Sauropsida, may have occurred at any time subsequent to the divergence of the synapsids from the ancestors of living reptiles and previous to the divergence of Prototheria and Theria. The extreme limits from all reasonable theories are rather closely prereptilian and post-cynodont or post-bauriamorph, but they are perhaps a little more likely to have occurred in the pelycosaurs than elsewhere.

The changes to a mammalian axis and atlas, and the development of a double occipital condyle, being related to head movement, are probably also related to increased activity. Although epiphyses were apparently absent throughout the therapsids, intervertebral cartilages seem to have been present in a cynodont (Haughton 1924). Epiphyses are also absent in *Oligokyphus* (Kühne 1956); it is uncertain how much before the Paleocene they appeared.

The presence of a diaphragm in some cynodonts and a therocephalian (Attridge 1956) is inferred from the great reduction of lumbar ribs (esp. Brink 1957a). This seems likely, and is an important part of their greater activity syndrome of characters. Brink's strange assertion that the cynodonts probably had visceral musculature, rather than somatic, in the diaphragm, contributing to their extinction, is unsupported (and it is more probable than not that at least some later mammals, triconodonts and multituberculates, quite possibly others, originated from this group).

The evidence for therapsid homothermy is derived from several sources. The general activity is perhaps the strongest indication, especially when taken together with their close relationship to conventional mammals, but the possible presence of complicated ethmoturbinals, sweat glands, and even hair, and an inference from pelycosaurs, are also suggestive. Redfield and an anonymous student (Romer 1948) have interpreted the enlarged neural spines developed independently in the pelycosaurs *Dimetrodon* and *Edaphosaurus* as providing a voluntarily modifiable surface for heat radiation and absorption from sunlight. If this increased regulation had developed twice in pelycosaurs, it is reasonable (although of course not obligatory) to suppose that it developed a third time (or was continued and increased) in their much more mammal-like relatives. Olson (1959) in fact thinks the therapsid radiation was probably initiated by the development of incipient homothermy. Schuh (1951) claims homothermy for many extinct reptilian groups, however.

The only major mammalian character group that does not seem to fit readily into any of the three adaptive categories proposed is the usual key complex, jaw-ear structure. The expansion of the dentary is, however, apparently related to activity by providing greater insertion for the adductor musclature, and Parrington (1934) and Watson (1912) have suggested that the reduction of the quadrate and posterior mandibular bones was related to this. It may be (as suggested by an undescribed cynodont of Patterson's) that sound was in this form conducted through the reduced articular and quadrate while these still functioned as the only or major jaw suspensorium. If this is the case, the advantage of a non-interfering articulation is clear. The apparent looseness of the small quadrate in the advanced bauriamorphs and cynodonts

suggests that firmer articulation may have been adaptively desirable even with a reptilian ear.

A few dozen further mammalian characters of lesser importance will be omitted; many of these also are know to have appeared in therapsids.

Not all typically mammalian (i.e. eutherian or, at most, therian) characters are present in all living mammals, as is well known, and the monotremes are on a more or less therapsid grade, although their brain is much more mammalian than reptilian. Little is at the moment known about most characters of Mesozoic mammals because of fragmentary occurrence, small size, insufficient searching in approporiate places with appropriate methods, and real scarcity in most preserved sediments. Some or almost all may have been on the prototherian grade in the sense of Thomas Huxley. In fact it is possible (Kühne 1956), but by no means demonstrated, that the adaptively relatively unimportant splenial persisted into the upper Jurassic in more than one mammalian group, which would make them reptiles by strict but unwarranted application of one of the four parts of the conventional key distinction.

Transfer of Therapsids

Thus we see that some therapsids appear to have possessed to a virtually mammalian extent the major ecological feature of mammals, increased activity. Intelligence is uncertain but apparently little more than reptilian in one case, although probably not a great deal less than most conventional Mesozoic mammals, and such speculations as can be made about the mode of birth and related features suggest that it was in some cases perhaps more likely to have been closer to typical mammals than to typical reptiles. Since advanced cynodonts and bauriamorphs, and to a lesser extent therocephalians and gorgonopsians, seem to have been much more mammalian than reptilian, it seems reasonable to consider them mammals rather than reptiles.

This, however, creates a practical difficulty in that the other therapsids form an obviously natural group with them, so much so that the origin of cynodonts from both the therocephalians (e.g. Broom 1938, Boonstra 1937, von Huene 1940, Brink 1951, Attridge 1956) and gorgonopsians (e.g. Watson 1920, Olson 1944, Romer 1956) is claimed by different authors because of different interpretations of the parallelisms involved, and the tapinocephalians are alternately grouped with the dicynodonts in the Anomodontia (Watson and Romer 1956) or with the titanosuchians in the Dinocephalia (Haughton and Brink 1954), to both of which they are manifestly related. All the therapsid groups show some tendencies in the mammalian direction, both independent and bequeathed from the sphenacodonts, and these are usually pronounced, although isolated reversals also seem to occur. Therefore the Therapsida as a unit may with justification be transferred to the Mammalia. To so transfer the sphenacodonts (and perhaps other pelycosaurs: see below) would be going beyond the first reasonable stopping place in the more reptilian level, and would in turn suggest transfer of the other pelycosaurs and perhaps even the captorhinomorphs (but see Parrington 1958), absurd steps in view of their quite reptilian grade. It is possible that the anomodonts and perhaps other less mammal-like therapsids should also be retained in the Reptilia, but this is not done here because of the increased difficulty at present of drawing a reasonably definable line.

Objections

There are several objections to this proposal. One, that not all therapsids were of mammalian grade, has just been dealt with. Related to this is the fact that intermediates are known between typical sphenacodonts and therapsids. These are now few, but may well increase

with more extensive exploration of the Ecca and Double Mountain equivalents. This is a taxonomically unfortunate but evolutionarily gratifying situation, which is at least equally true with the jaw-ear criteria. There are now known four genera, or probably more, some or perhaps even all of them unrelated, that had or very likely had a double jaw articulation: *Docodon* (and perhaps its relative *Peraiocynodon;* Kühne 1958, Kermack and Mussett 1958b), *Morganucodon* (perhaps same as *Eozostrodon;* Kühne 1958, Kermack and Mussett 1958b), *Diarthrognathus* (Crompton 1958), with possibly also an undescribed symmetrodont (Kermack and Mussett 1958a). The tritylodontid *Oligokyphus* may well have been in a similar situation. Plate VI, figure 2 of Kühne (1956) shows a small glenoid fossa in the squamosal, and Mr. Giles MacIntyre has pointed out to me that the apparently pointed condylar process of the dentary is probably merely broken, as can be seen from a careful examination of Plate V, figure 1. No clear demarcation exists between a number of higher taxa, as is well known. The classes Reptilia and Amphibia merge insensibly in the seymouriamorphs, and the protoungulate order Condylarthra is absolutely continuous with the Creodonta as presently defined (e.g. Gazin 1941; Simpson 1936, 1953). In such cases, as here, it must be arbitrarily decided which criteria are to be given most weight. Adaptation is chosen here.

Perhaps more serious are the objections of tradition, pedagogic convenience, and definition. An established boundary should not be changed without good reason. Probably all current texts that consider fossils place the dividing line in accordance with the jaw-ear criteria. And by the change the definition of the class Mammalia becomes less clearcut, although more significant ecologically.

But there does seem to be good reason for the change; if this is agreed on texts will change, and if it is not this paper has failed in one of its purposes. Many taxa lack unambiguous definitions that include all that should be included and exclude all others. Even in the present case good definitions based on characters of great adaptive significance can be framed directly from the previous discussion to include all mammals except some of the therapsids; many current neontological definitions would probably include some of them. A completely limiting definition is perhaps as follows: Synapsid tetrapods with (1) a small quadrate, (2) no supratemporal, and (3) a phalangeal formula functionally 2,3,3,3,3 (secondarily increased in cetaceans; additional disc-like phalanges to the reptilian quota present in some therapsids). The second and third criteria are not known in many instances; their use here is permitted by our ignorance. The first, on the other hand, is usually well known but difficult to define objectively. When borderline cases are better known one or more of these may have to be omitted.

The present allocation also may make the mammals monophyletic in origin or nearly so on the basis of present evidence, having arisen from perhaps a single family. Undescribed Russian material seen by Olson (*in verbis*) suggests to him, however, that the dicynodonts may have been derived from the edaphosaurs and that further polyphyly is possible. In addition, it will perhaps be easier to tell whether or not a group or specimen is to be considered mammalian, although this is uncertain.

Classification

There is (I hope!) unanimity that the Theria should be included in the Mammalia and that the Sauropsida of Goodrich (1916) and Watson (1957) should be excluded. Disagreement is possible on the remaining groups. G. MacIntyre (*in verbis*) feels that the term Mammalia should be restricted to the Theria and other traditionally mammalian groups left *incertae sedis*. This possibility

is also suggested, but not adopted, by Simpson (1959). Another possibility is to group all Goodrich's theropsids with the Mammalia, as he in fact suggests might become desirable, and as Kuhn (1959) proposes. If, however, grade of adaptation is to be made the primary basis for placement, the pelycosaurs and other non-therapsids must be excluded. The next to limiting possibility of vertical classification, inclusion of the Mammalia as a suborder of the Therapsida, has actually been proposed by von Huene (1948, although not elsewhere, e.g. 1956). Transferral of the Therapsida and only the Therapsida to the Mammalia has to my knowledge not been previously proposed in a published paper, although it has been suggested as an alternative solution (Simpson 1959). The group has been placed among the Reptilia because many of the more obvious skeletal features are reptilian and because most of the earlier known forms were less mammal-like than many now known. The features upon which the present proposal is made are often rather recondite in themselves, though indicating features of great adaptive significance. Accepting or rejecting this suggestion is dependent to a large extent on weighting of conflicting criteria by one's own tastes.

Upon the inclusion of the therapsids in the Mammalia (as the "reptile-like mammals"), it is necessary to decide how to place them there. Formal proposals are best postponed until the origins of the various other mammalian groups from the therapsids are better established. It is however probable that a split of the Mammalia into two subclasses having respectively the Therocephalia and Cynodontia as basal members may eventually be desirable, whether or not the present transfer is accepted, with perhaps a third for the remainder of the therapsids. This combines a horizontal taxon for a basal radiation with vertical taxa for more successful groups, as is a common practice elsewhere. Provisionally the Therapsida may be left as a basal subclass and expanded to include forms of uncertain affinity: the Haramiyidae, Ictidosauria, Tritylodontidae, Docodonta, eozostrodonts, "protodonts," and perhaps the Triconodonta.

Summary

It is maintained that the most important criteria for separating tetrapod classes should be their major adaptive differences. Evidence is presented to indicate that the Therapsida were probably more mammal-like than reptile-like in this respect, and therefore the Therapsida are transferred to the Mammalia. Objections, alternate possibilities, and the resulting reclassification of the Mammalia are considered.

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POLYPHYLETIC OR MONOPHYLETIC ANCESTRY OF MAMMALS, OR: WHAT IS A CLASS?

CHARLES A. REED

College of Pharmacy, University of Illinois, Chicago, Ill.

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A recent issue of EVOLUTION carried two articles (Olson, 1959; Simpson, 1959) on the origin of mammals. Olson looked up, as it were, from the murky depths of the Pennsylvanian and saw the ongoing evolution of the Synapsida culminating in the late Triassic in several lines which he could call mammals. Simpson adopted the opposite point of view, and looked down the mammalian evolutionary sequence from the end of the Mesozoic. However, he arrived at the same time-level and the same conclusion as did Olson: The mammals are polyphyletic, and the several lines involved (possibly nine, probably no fewer than four) independently crossed the reptilian-mammalian boundary in the late Triassic or near the division between the Triassic and Jurassic. Simpson wrote further, "As far as I know, all subsequent authors who have explicitly studied the Mesozoic mammals have agreed that they were polyphyletically derived from reptiles. . . ." Olson stated essentially the same (p. 348), although he did mention that a few students disagreed. However, the paleontologists, at least, are in almost total agreement.

Simpson, with his usual prescience, proceeded to clarify the possible implications, in terms of formal taxonomy, of the meaning of the words 'Class Mammalia' if the present conclusions as to polyphyletic origins of the class are considered valid. Of various alternatives presented (p. 413) he personally preferred his third, "The groups known to have reached mammalian grade (by whatever of the diagnoses one prefers) may be retained as a phylogenetically based Class Mammalia. . . ." By 'phylogenetically based' he means ". . . arose wholly from one of lower categorical level, as Class Mammalia from Order Therapsida."

As matters stand at present, then, several different groups within the Class Mammalia (as commonly accepted) are related to each other only by descent through several groups of therapsid reptiles. Such a concept of the meaning of 'Class' and of 'mammal' will be strange to most zoologists, trained as they have been in the tradition of monophyletic descent of all members of any taxon.

Simpson does, indeed, suggest the possibility of a logical monophyletic beginning for all mammals by shifting the origin of the class backward in time to a common ancestor (his alternative 4, p. 413): "Some or all of the animals now called mammallike reptiles may be included in the Mammalia." The consequences of such a decision would obviously complicate the work of the paleontologists by changing the bases for differentiating between mammalian and reptilian fossils to different, even if more indefinite, characters than now used. Also, I suspect, such a decision would be distasteful to their present feeling of the proper order of things. Furthermore, continued Simpson, ". . . such a radical change would make all current and earlier literature confusing and would greatly complicate teaching and popularization."

Perhaps it is time that one who has not ". . . explicitly studied the Mesozoic mammals . . . ," and thus one not hampered by the necessity of identifying specific fossils, had an overall look at the problem. Upon doing so, several

conclusions and present practices are obvious. The practices are:

1. The general subject of the origin of mammals has necessarily been one for paleontological exploration and interpretation.

2. Paleontologists studying Mesozoic mammals have available only bones and teeth, and so naturally make their identifications and draw their phylogenetic conclusions on the basis of the osteological evidence available to them, although not ignoring the logical probabilities involved in the evolution of the other body systems.

3. The 'first mammals,' as presently defined, are identified on the basis of details of the lower jaw-middle ear complex, with the choice of the determining detail varying according to investigator (Simpson, 1959: 407–408). Simpson and some others, as a "practical criterion," choose the presence of a dentary-squamosal joint as the osteological character by which a mammal is to be defined. Other workers would choose one or several other details as being essential, but the main factor—that of an arbitrary choice of details of one functional osteologic complex—is otherwise the same.

The following conclusions, as now known, are agreed by all:

1. Several lines of the Therapsida became progressively more 'mammallike' in a number of osteological and dental characters.

2. Of these several lines, some few (the exact number has no significance to the present discussion) independently achieved a dentary-squamosal joint, and are thus called mammals by Simpson and others. (If one demands other lower jaw-middle ear changes as necessary to define a mammal, fewer groups of 'first mammals' will result, but these are differences in detail and not of basic principles.)

We are thus, once again, faced with the problem of "What is a mammal?" Obviously, if we follow any definition based on any combination of lower jaw-middle ear characters, the mammals were polyphyletic, as Olson and Simpson and other paleontologists point out in detail, and the matter is closed.

If we consider the living mammals, however, we find that the individuals of the two surviving subclasses (Prototheria and Theria) have the following characteristics in common (the first two are also present in birds, where they were independently evolved and differ in detail, but this independent evolution does not concern us here):

A. Non-skeletal characters:

1. Endothermy.[1]
2. Complete double circulation, with higher arterial pressure in the non-pulmonary portion.
3. Retention of the 4th left aortic arch as the functional arch of the aorta.
4. Enucleated, round erythrocytes (secondarily oval in the Camelidae).
5. Loss of the renal portal system.
6. Possession of a diaphragm.
7. A combination of integumental characters: hair, sebaceous glands, and sweat glands.
8. Possession of mammary glands, functional in the females.
9. Nitrogenous wastes excreted as urea instead of uric acid.
10. Possession of specialized facial dermal muscles.

B. Skeletal characters:

1. Articular-quadrate joint not a suspensorium.
2. Dentary-squamosal joint present.

[1] I agree with Cowles and Bogert (1947) that the use of 'endothermy' and 'ectothermy' is preferable to the more usual 'homoeothermy' and 'poikilothermy.'

3. Three middle ear ossicles present.
4. Mandible consists of one bone only, the dentary.
5. Secondary (false) palate present.
6. Double occipital condyle instead of but one.
7. Mammalian-type atlas-axis complex.
8. Cusps present on the cheek-teeth.
9. Lumbar ribs lacking.
10. Mammalian-type ethmo-turbinals present.

Do those who advocate a polyphyletic origin of mammals intend to imply that the totality of this rather imposing list of functional and morphological characters was evolutionarily duplicated several times? There is a danger that some unwary readers might so believe, but the paleontologists involved are much too knowing of the total biological pattern ever to have had such a naive idea. These paleontologists realize: 1) Many of these characters were undoubtedly present in many of the Therapsida and possibly in some of the Pelycosauria, but left no traces in the fossils; 2) Some of the non-skeletal characters *may* actually have originated and evolved separately in individual lineages of therapsids, as has been determined for several of the osteological complexes.[2]

From these observations on the independent evolution of skeletal complexes Olson draws the conclusion, completely correct I believe, that the evolving osteological characters available to paleontological study are merely secondary evolutionary manifestations of more fundamental physiological changes—namely, endothermy and its correlated greater bodily activity.

Thus, the osteological characters of the lower jaw-middle ear complex upon which the paleontologists have based the answer to "What is a mammal?" are secondary results of more profound and much earlier evolutionary sequences. Olson saw this clearly, but then rejected what to me is the logical taxonomic conclusion—namely, that the animals that possessed the primary modifications (most probably physiological, as he himself pointed out) are themselves mammals. In other words, I must agree with Simpson's alternative 4 (p. 413), that "Some or all of the animals now called mammallike reptiles may be included in the Mammalia," except that I would substitute 'should be.'

The essence of the difference of opinion between myself and those who hold for a polyphyletic origin of mammals lies not so much in the answer to the question of "What is a mammal?," but of "What is the fundamental basis for establishing a major taxonomic category?"

Any group of animals of major taxonomic status (phylum, class, order, family) has undoubtedly had its beginning due to the establishment of some particular adaptive trend,[3] the accentua-

[2] The pattern of such independent origin and parallel evolution of complexes of osteological characters in independent lines of therapsids must force a re-assessment of our traditional thinking in comparative anatomy. For instance, if we had only living mammals to study, we would have assumed that as intricate a mechanism as the mammalian lower jaw-middle ear complex would have evolved *in toto* only once, and thus necessarily have been present in the common ancestor of all living mammals. However, substantial and increasing evidence indicates the contrary. Indeed, it would appear that most, if not all, of the skeletal characters listed above were evolving toward a 'mammallike' condition in different therapsid lines.

[3] I do not mean that the adaptive trend was necessarily determined by a single mutation; the situation in each instance was undoubtedly much more complex. I do not mean that the ancestral form had the adaptive character or characters completely developed; indeed at first these characters would be most incipient. I do not mean that species or genera closely related to the ancestral form might not also possess some aspects of those particular adaptive characters which ultimately led to the 'biological success' of the taxon as a whole;

tion of which has set the pattern for the whole subsequent evolution of the group and been the initiating factor in the appearance of numerous secondary and then tertiary combinations of characters which serve to separate the subgroups as they appear. One should, then, attempt to discover the primary adaptive changes that mark the group as a whole, and not use as criteria subsequent characters which may be true of only certain of the sub-groups.

With the mammals these primary characters, as pointed out so clearly by Olson,[4] are undoubtedly those associated with the tendency toward endothermy and thus the original mutations may well have had to do with increase in efficiency of intracellular oxidative enzymes. Such physiological changes, accompanied by separation of the pulmonary circulation from the body circulation, would probably have been sufficient to initiate the endothermal tendency toward "mammalness."

By all the presently-available paleontological evidence, and according to present nomenclature, the bones of such an animal that had these basic biochemical and circulatory changes belonged to a reptile—Olson named the group directly; it would have been one of the more advanced of the sphenacodont[5] pelycosaurs of the early Permian (fig. 1). If, 1) that group of pelycosaurs was the common ancestor of all mammals and if 2) as seems probable, they already had the basic mammalian 'invention' that led to the ultimate dominance of the group, is it not then logical (even if difficult proctically, as of the present) to include this common ancestor within the class it initiated? Thus these sphenacodont pelycosaurs and all of their descendants would be mammals. The mammals, by this standard, are monophyletic, and the class would clearly be regarded as a clade and not as a grade (Huxley, 1958).

The difference between taxonomies based upon the concepts of the 'clade' and the 'grade' is essentially the crux of this whole argument. As discussed by Huxley (1958) a clade is a monophyletic unit of any degree, from morphism to kingdom; clades thus represent the products of evolutionary divergence (cladogenesis). A grade is a unit which results from thinking about evolution in quite a different way; a grade is a group which has acquired certain characters in common as a result of evolutionary 'improvement' (anagenesis). A grade may include two groups related only distantly through a third group (i.e., the grade Homotherma of birds and mammals, having the common character of endothermy), or may set apart at the horizontal level within a single major radiation, those subgroups which have independently acquired some particular character (i.e., those therapsids which possess a secondary palate).

Huxley stated quite clearly (1958, pp. 26–27) that the customary taxonomic terminology applies to clades (i.e., monophyletic units, as I am arguing) and that quite a different terminology, involving concepts of degrees of evolutionary specialization (however achieved), has to be

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it is however the persistence and accentuation of the trend in the successful group through a considerable time, contrasted with (usually, at least) the extinction of the closely related lineages, that mark the major taxon. I state these matters explicitly, as each has been misunderstood by some with whom I have discussed these problems.

[4] I certainly, and Olson, I strongly suspect, owe a considerable intellectual debt to Brink (1956). Certainly many of the ideas here expressed have been formulating slowly in my own mind since reading his stimulating paper several years ago.

[5] Future research may show, according to the views expressed by students of primitive reptiles at the 1958 annual meeting of the Society of Vertebrate Paleontology, that not all the Therapsida were derived from any one group of pelycosaurs (as that order is now defined). Naturally, further knowledge will be expected to clarify exact relationships, but cannot (insofar as I can see) negate the principles I am discussing.

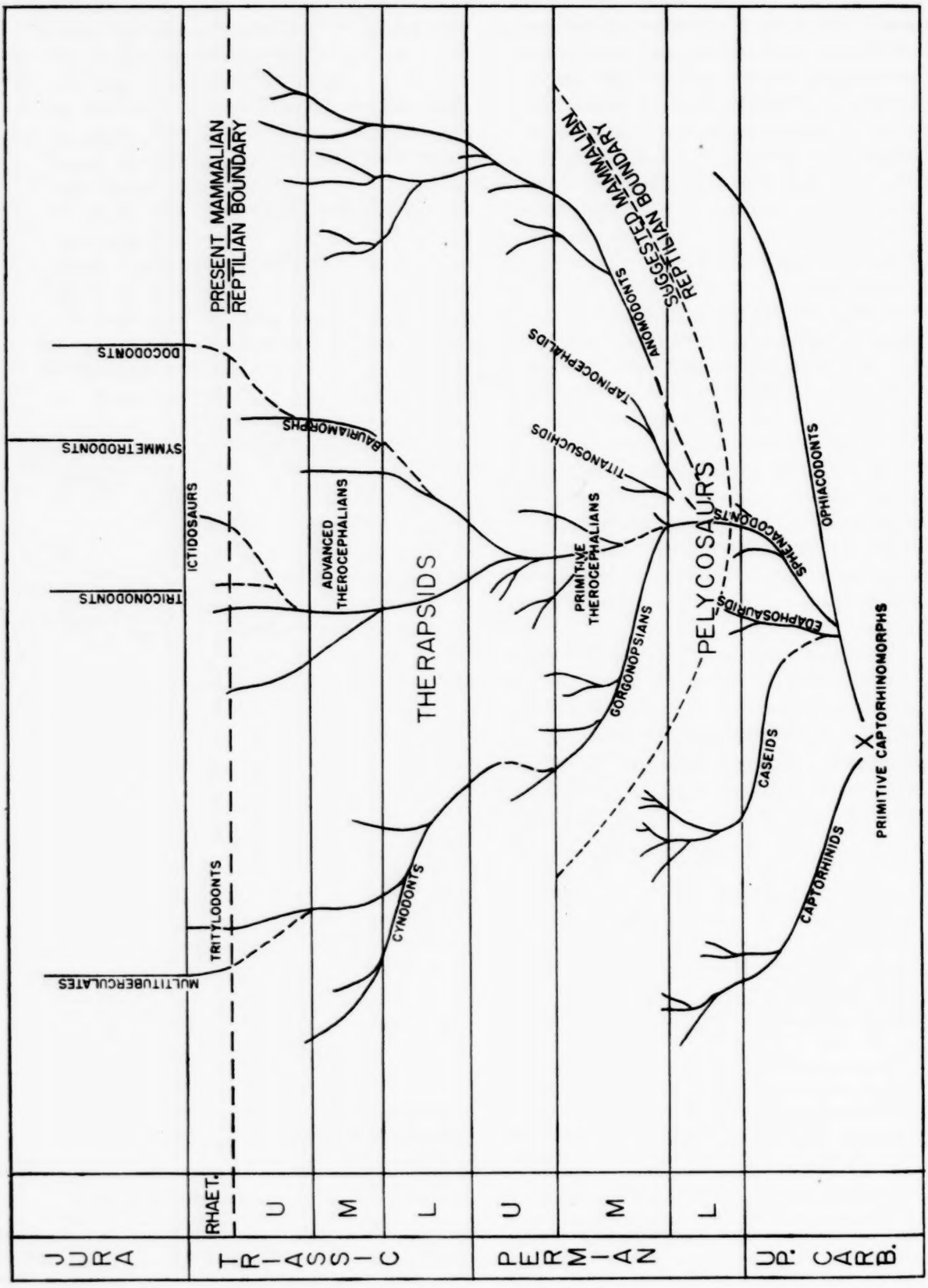

FIG. 1. Evolution of mammalian predecessors: Present view contrasted with that presented in this paper. (Chart adopted from Olson, 1959, by permission.)

used for the units he formalizes as grades. Indeed, it is the difference in concept between clades and grades, and the impossibility of including both types of thinking within the expression of a single formal taxonomic system, that underlies Huxley's whole article. He so stated (1958, p. 27): "I accordingly suggest that the customary terminology purporting to define phyletic units should be *supplemented by a secondary terminology* aimed at delimiting steps of anagenetic advance." (Italics mine.) He admitted that many authors have advocated the use of grade where the phylogeny is obscure, but demurred at this mixing of categories of classification (p. 29): "However, as will have become clear, the facts of evolution make it necessary to envisage a two-way system of classifying organisms in general, by anagenetic level as well as by cladogenetic (phyletic) divergence, . . ."

Expressed in other terms, the argument essentially is the old one between classification based on vertical principles (by clades) and horizontal principles (by grades). It is quite true that, considered in time-depth, horizontal classification is always more convenient. Vertical classification typically results in removing one adaptive line from a number of evolving lines of an adaptive radiation and including that one line in another taxon for the reason that the one line differed from its brother-lines by having a potentiality that led to a major new taxon. Vertical classification in practice is thus generally most inconvenient to the working paleontologist.

Biologists have never formally decided upon which principle classification is to be based. Generally, it seems to me, they have talked (and perhaps thought) they were adopting a vertical scheme, based on clades, but instead have generally, even if unconsciously, settled for some degree of a horizontal classification, based on the more convenient grades. As Huxley explains, however, the two systems are founded on different kinds of concepts, and require different sets of terminologies, but at present most of us are confusing the concepts by trying to envelop both systems within a single kind of terminology.

If, as Huxley says, the "customary terminology" should be applied to clades, then a different vocabulary, as yet nonexistent, must be invented for classification by grades. Another possibility, of course, is for biologists to agree formally to classify horizontally, by grades, and invent a new terminology for the truly different vertical system, based on clades.

Logically, there is no middle ground, but our taxonomic principles now wander in that illogical middle ground, and will probably remain there for some time.

However, I agree with Huxley that the expression of the phylogeny is the important matter, and that the customary taxonomic terminology does, or at least ought to, apply to clades. Thus a class or any other taxon, it certainly seems to me, should always ideally be regarded as a monophyletic unit and thus it should be logically impossible to speak of the polyphyletic origin of any taxonomic group. Indeed, only if and when biologists in general come to agree that the customary taxonomic units shall *not* apply necessarily to clades (monophyletic units) but shall be applied *instead* to grades (units with similar degrees of similar specializations) can we speak or think of anything but monophyletic origins in the usage of our "customary terminology" in taxonomy. The burden of proof would thus seem to be upon those who wish to define any class or any other taxonomic unit as a grade; they must first convince biologists in general that taxonomy should be based upon similarity of degrees of specialization and not upon the fact of a common origin.

Aside from the logical necessity of regarding a class as the whole of a monophyletic unit, if we think of the Class Mammalia as monophyletic and as including its own basal stem within

Fig. 1. Evolution of mammalian predecessors. (From [illegible] 1959, by permission.)

itself, we concentrate attention upon the *original* similarities and the fundamental adaptive trends of the group as a whole. In contrast, the present polyphyletic scheme—at least to most biologists, even if not to specialists of the Synapsida and of Mesozoic mammals—presents a disrupted pattern of independent evolution of several partially-related groups which somehow became similar enough to be called mammals. The fundamental probability that the similarities were actually due to parallel evolutionary development, in several therapsid lines, of trends initiated in a common ancestor are either ignored or strongly subdued—by the very act of putting the common ancestor into a different vertebrate class—by those who argue for a polyphyletic origin of mammals.

There is increasing evidence as we learn more of the therapsids that in behavior many of them are probably as mammallike, if not more so, as they were reptilelike (Brink, 1956, 1959; Colbert, 1958). There is indirect evidence for the presence of a diaphragm, and one suspects that they were hairy and had functional mammary glands. Locomotor adaptations indicate that they were active, non-sprawling animals capable of relatively (for the period) high speed, and their dental characters indicate that they chopped and/or mashed their food, as an aid to rapid digestion and resultant quick release of energy.

In these types of activities, and in the anatomical and physiological adaptations correlated with them, the therapsids were—so it seems to me—more mammalian than reptilian. If additionally we can find evidence that they had hair (as Brink believes may be possible) then it seems to me that it will be difficult to maintain that they should be retained in the Reptilia, even for those to whom the logical necessity of monophyletic classification is not important.

Paleontologists, of course, do not deny that the different groups they call mammals had an ultimate common ancestor somewhere in the synapsida. Their argument for maintaining the presently-accepted definition of the Class Mammalia, with its necessary polyphyletic consequences, includes then more than the acceptance of a 'practical criterion' of a single osteological character. Additionally, consciously or unconsciously, they must agree with a philosophy that results in the taxonomic principle that a grade can be a formal taxonomic unit, as clarified by Simpson (1959: 413) in his alternative 3.

In the same paragraph Simpson rejected the taxonomic principle that would lead to the concept favored in this paper —that of a monophyletic origin for the class. He wrote: "It is always necessary in a formal classification (so unlike an actual phylogeny) to compromise sooner or later between horizontal and vertical separation of taxa. That each taxon sometime included only one species in its ancestry should be true, but it is a completely impractical requirement that each taxon be so delimited and defined as to include and begin with that species. In practice it is a sufficient principle for evolutionary taxonomy that each taxon arose wholly from one of lower categorical level, as Class Mammalia from Order Therapsida." Additionally, he has further emphasized the same ideas in personal correspondence: ". . . when you did get down to the single species or genus that you are seeking in order to make the taxon truly monophyletic in the very narrowest sense, you would find that there were closely related genera of the same family or closely related species of the same genus that were not ancestral to the mammals. You would then, in all logic, have to refer two species of the same genus or two genera of the same family to two different classes. That obviously is not practical and in the exigencies of the classificatory situation you simply have to seek some different solution."

The facts are self-evident, but I cannot agree with the argument or the conclusions, as such natural but unfortunate separation of closely-related genera must necessarily prevail in any biological continuum where lines are drawn between branches of the evolutionary bush. (Such separation of closely related genera does of course already exist at the presently-accepted Reptilian-Mammalian boundary, particularly in the tritylodonts.) Since such separation of related genera into different classes must necessarily occur, it would seem preferable to have to make the difficult decision at one locus in time and evolutionary sequence, and not for the several separate loci (four or more) demanded by the present polyphyletic scheme.

Eventually, of course, the decision concerning which genera are to be included in which of two classes will be an arbitrary one, and will depend upon the measured judgement of a few specialists. The *kind* of arbitrariness which will be exercised here, however, seems to me to be necessary, unavoidable, and fundamental, and thus in a totally different category from the arbitrary decision of present paleontologists that the origin of the Class Mammalia is to be defined on the basis of one skeletal character.

Biologists have not in the past allowed matters of tradition or convenience to stand in the way of major taxonomic revisions when increasing knowledge demanded change. (Otherwise, we would have with us yet the phyla Vermes and Radiata!) More recently, and within the Mammalia themselves, the traditional, easily teachable, tri-partite sub-ordinal division of the Rodentia has been abandoned (Wood, 1959: Wood and Patterson, 1959) resulting in a confused and continuously changing picture that is hardly teachable and certainly not capable of popularization. The authors involved, however, did not hesitate on these grounds, but hewed to the line.

On the other hand, biologists have certainly not in general delimited major groups by convenience of definition, when logic dictated (as I believe it does with regard to the supposed polyphyletic ancestry of the mammals) that the convenient criterion resulted in major error.

Acknowledgments

I am indebted to a number of vertebrate paleontologists, particularly G. G. Simpson of The Museum of Comparative Zoology, to E. C. Olson of the University of Chicago, and to Rainer Zangerl and William Turnbull of The Chicago Natural History Museum, for illuminating comments on this manuscript. None of them agree with my conclusions, but their thoughtful disagreements have helped clarify my own views.

Summary

1. A number of students, mostly paleontologists, have published in recent years on the supposed "polyphyletic origin of mammals."

2. In each such instance the definition of mammal has been based upon possession of one or more details of the lower jaw-middle ear complex. The Class Mammalia, as thus defined, is a grade (a group with a similar degree of anagenetic specialization) and not a clade (a monophyletic unit).

3. However, all of the animals thus defined as mammals *do* have a common ancestor, which would at present be regarded (Olson, 1959) as one of the sphenacodont pelycosaurs of the early Permian. Alone amongst the pelycosaurs, these sphenacodonts show incipient mammallike characters.

4. Olson has discussed the illuminating probability that the basic mutational changes which led the synapsids toward 'mammalness' were physiological, without at first morphologic representation (at least in so far as can be observed on the known fossils). Later, independent and convergent evolution of various skeletal

complexes to a condition at present defined as 'mammalian' was probably channelled toward similar or the same ends by similar selective forces acting upon similar gene-complexes, with the physiological factors discussed by Olson being, in a way, 'pre-adaptive.'

5. The basic physiological changes (very probably some degree of endothermy and increased bodily activity) undoubtedly occurred in the common ancestor of the Therapsida; this common ancestor of the Therapsida was also the ultimate common ancestor of the mammals (however defined).

6. Any taxon (CLASS MAMMALIA in the present discussion) should correspond as closely as possible to the single phyletic, evolutionary unit that has actually existed in time. Thus a taxon should be a clade (whenever determinable) and not a grade (Huxley, 1958).

7. Zoologists in general, then, should seriously consider the logical inference that the Therapsida and those pelycosaurs immediately ancestral to them be removed from the Class Reptilia and placed in the Class Mammalia.

8. The result of the suggested change would be a natural taxonomy and monophyletic origin of the Class Mammalia.

9. The presently-accepted definition of CLASS MAMMALIA represents a horizontal concept of classification, based on the principle of the grade (Huxley, 1958). Such a classification can only partially express phylogeny and will inevitably result in polyphyletic patterns.

10. Classification systems based on both clades and grades can exist, but should be kept separate since one terminology cannot describe the results of quite different kinds of evolutionary events.

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THE ENDEMIC FISH FAUNA OF LAKE LANAO, AND THE EVOLUTION OF HIGHER TAXONOMIC CATEGORIES [1]

GEORGE SPRAGUE MYERS

Stanford University

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INTRODUCTION

The present paper is concerned only incidentally with speciation. Its purpose is to point out some striking but neglected features of lake-fish evolution that illustrate the rapid origin of genera and still higher categories going on at the present time. I have selected the Lanao fishes as an example for several reasons. First, I have examined the fishes myself. Second, the Lanao fishes are in a recognizable stage of what has been called "explosive" evolution. Third, the age of the lake can be determined geologically, and its relative youth cannot be in serious dispute. Fourth, the remarkable zoogeographical situation of Lanao and of Mindanao Island excludes any reasonable possibility that more than one still existing species could have given rise to the 18 endemic species and four endemic genera now inhabiting the lake. The endemic Lanao fish fauna is without parallel, so far as known, in demonstrating explosive specific and generic evolution from a known and still existing ancestral species.

I am deeply indebted to my long-time friend and colleague, Dr. Albert W. Herre, discoverer and describer of the Lanao fish fauna, for many discussions regarding Lake Lanao and its fishes, extending through a period of 30 years. The late Professor Bailey Willis of Stanford, well known for his geological researches on four continents, did me the honor of employing his extensive first-hand knowledge of Philippine geology to prepare the brief geological account of Lake Lanao quoted below. Finally I must present my best thanks to my present graduate student, Mr. Angel Alcala, Instructor in Zoology at Silliman University, Dumaguete, Oriental Negros, for making further collections of Lanao fishes for Stanford. Mr. Alcala was working under National Science Foundation Grant G4381, made to Dr. Walter C. Brown for herpetological research in the Philippines.

THE LAKE

Lake Lanao lies at an altitude of approximately 2,100 feet in the midst of a volcanic area in central Mindanao, the largest island of the southern Philippines. Its exact area is in dispute, Herre giving it 375 square kilometers and others as many as 900. The late Professor Bailey Willis, who had given much attention to Philippine geology, as well as to that of the African Rift Valley and its lakes, investigated what was known of the geological history of Lake Lanao and prepared the following statement for me:

"The island of Mindanao has risen from the ocean gradually and unequally since the Miocene. It now consists of plateaus, hill country, swamps, and volcanoes. The streams were initially small and isolated from each other. The headwaters were, and are, generally swift and the lower courses estuarine.

"A north-central region was built up by basalt flows to a plateau, on which a small system of rivers developed. Some of them flowed southwesterly to Illana Bay, others northwesterly to Iligan Bay. The divide between them ranged from southwest to northeast.

[1] Abstract published in: Proc. XVth Internat. Congr. Zool., 151–152, 1959.

"Volcanoes were built up across the southwesterly flowing streams and they were dammed. Their headwaters gathered in the basin thus formed until they overflowed a low pass in the divide at Camp Keithly and discharged into Iligan Bay on the north coast.

"The impounded waters constitute Lake Lanao. The basin is probably shallow, 200 to 300 feet, perhaps, where deepest. The outlet at Camp Keithly plunges over a fall into a short canyon, indicating an age of 10,000 years, more or less. The principal tributaries to the lake enter from the southeast, from young but dormant volcanoes, and may at times have brought in quantities of ash."

While I have no specific reason to doubt Dr. Willis's estimate of the age of Lake Lanao, 10,000 years seems to be a very short time for the evolution of the Lanao fish fauna. When I brought up this question, Dr. Willis replied that the length of the canyon worn by the Agus River indicated a very brief erosional period. The possibility remains that more than one volcanic damming has been involved in the history of Lake Lanao, but the relative youth of the lake cannot be seriously doubted. The geology of the Lanao Plateau obviously needs more geological investigation.

The Fishes

Dr. A. W. Herre collected fourteen species of the Lanao fishes and described them formally in 1924, without mentioning the peculiar evolutionary features concerned. Before the publication of his 1924 paper, he had prepared an account of the zoogeography of Philippine freshwater fishes, in which he implicitly recognized the autochthonous nature of the Lanao fish fauna. However, this distributional paper was not published until considerably later (Herre, 1928). Dr. Herre visited the lake upon later occasions, adding two more species in 1926 and two in 1932.

After Dr. Herre joined the Stanford Museum staff in 1928, I urged upon him the value of preparing an account of the evolutionary features of the Lanao fauna. This resulted in the proposal of a new genus for one remarkable Lanao species (Herre and Myers, 1931) and, finally, in Herre's well-known paper of 1933. Since that time, nothing of importance has been published on these fishes save for Dr. Brooks' review of 1950.

This history is important because of the destruction of Herre's earlier material when the Bureau of Science was dynamited and its collections totally destroyed by Japanese troops in February, 1945, during the battle of Manila. The only sizable collection of Lanao fishes presently available is that in the Natural History Museum of Stanford University. This consists of a few specimens obtained by exchange from the Bureau of Science before World War II; the excellent collection made by Dr. Herre in 1931, including the types of two of his species; and a small collection made at my request in 1959 by Mr. Alcala. Two of the endemic genera (*Mandibularca* and *Spratellicypris*) and the majority of the species are represented. The only other collection of Lanao fishes known to exist in any museum is a small one obtained in 1908 by Dr. Hugh M. Smith and Dr. Paul Bartsch, and now in the U. S. National Museum. This collection was not reported upon *in extenso* until long after Herre's work was completed (Fowler, 1941). I am unable to accept some of Fowler's identifications and have not considered them in the present paper. Two of Herre's endemic genera (*Cephalakompsus* and *Ospatulus*) are known only from the destroyed types.

I have examined the Stanford collection and have had much unpublished information from Dr. Herre. Despite the unavailability of several of the described species, I am convinced that most if not all of the species described by Herre are distinct, some of them remarkably so. Unfortunately, little ecological information

is on record. Most of the collections obtained from the lake have been purchased from the native fishermen. The lake is extensively fished, and some of the endemic forms are highly prized as foodfishes by the local Moros.

The endemic forms are all members of the Cyprinidae, the largest family of primary fresh-water fishes. The large species flock consists of 13 known species of the genus Dr. Herre called *Barbodes*, better called *Puntius* (see Weber and de Beaufort, 1916) but in my opinion not easily distinguished from the widespread genus *Barbus* (Myers, 1960). Five more species are placed in four genera, *Spratellicypris* (1), *Mandibularca* (1), *Ospatulus* (2), and *Cephalakompsus* (1), all of them obviously immediately derived from stocks of *Barbus* within the lake. Other still undescribed species are probably present in the lake. Two non-endemic predators are present, *Channa striata*, perhaps introduced by man, and one diadromus eel (*Anguilla celebesensis*). Eels of this group are known to be able to ascend rapids and waterfalls impassable to other fishes. The North American black bass (*Micropterus salmoides*), a voracious predator, is said to have been introduced in recent years.[2]

Derivation of the Mindanao Cyprinidae

Herre (1928 and 1933) has outlined some of the distributional history of the Philippine Cyprinidae, and I have published a general study of the zoogeography of the fresh-water fishes of the region (Myers, 1951). The essential facts are as follows:

Central and southern Borneo teems with Cyprinidae, but the cyprinid fauna of North Borneo is relatively depauperate. Cyprinids have entered the Philippines from North Borneo in two widely different directions, through the Palawan-Calamianes chain to Mindoro, and through the Sulu chain to Mindanao. Cyprinids got no farther. The family is absent in the rest of the Philippines, and in Celebes. The Palawan-Mindoro cyprinids do not concern us here.

That Cyprinidae reached Mindanao via a sweepstakes route, across a series of salt-water gaps, is unlikely. My own studies (Myers, 1938, 1949, 1951) indicate that fresh-water fishes are less likely to cross such gaps, especially a series of them, than any terrestrial animals, although they must have done so (probably only once, across a very narrow barrier) at Lombok Strait (Myers, 1951). The Lombok crossing, if not by the hand of man, was almost certainly by means of a local cyclone (Darlington, 1938; Myers, 1951), for the salt-water gap at Lombok Strait, although probably broader now than in the Pleistocene, cannot have been bridged very recently (see Bruun and Kiilerich, 1957). Nor is it likely that hurricane (typhoon) winds could have aided the fishes invading Mindanao. The typhoon tracks shown by Dickerson (1928: 40) are all westerly in direction. Finally, fresh-water fishes are not well adapted to raft-dispersal across seas!

The obvious conclusion is that fresh-water fishes entered Mandanao across a dry-land filter bridge, through the Sulu chain. Just what lowering of sea-level occurred there during the Pleistocene, or what elevations or depressions of the Sulu chain may have occurred, is not known. The region is a volcanic, unstable one.

That few or no remains of the cyprinid migration are to be found today on the islands of the Sulu Archipelago is not too surprising. Dr. Herre fished the largest island, Jolo. He found the streams small and without Cyprinidae, but believes that relatively recent volcanic activity has wiped out the fresh-water fishes of the island (Herre, 1928).

[2] It is difficult to see why such an introduction should have been considered. Ecologically, and as a measure for increasing food production, it is clearly unsound. Scientifically, in view of the unique nature of the endemic Lanao fish fauna, it becomes a crime!

Quite clearly, then, North Borneo itself, together with the Sulu Archipelago, acted as a filter bridge to limit the access of fresh-water fishes to Mindanao. Only three genera of Cyprinidae reached Mindanao (*Barbus, Rasbora,* and *Nematabramis*) and these three are still the dominant cyprinid genera in the streams of North Borneo. Probably only one species of each genus reached Mindanao.

The cyprinid fauna of Mindanao Island outside the Lanao Plateau is very small. There is a single endemic *Rasbora* (*R. philippina*) confined to the western part of the island and closely related to a North Borneo species (Brittan, 1954: 127–131). There are two species of *Nematabramis* (*N. alestes* and *N. verecundus*), very closely allied to each other and to the species of North Borneo (Herre, 1953). Finally, there are four nominal species of *Barbus* (or *Puntius*). *Barbus binotatus* is widespread in Mindanao, and, according to Herre (1953: 123), has been erroneously reported from Lake Lanao by Fowler. *Barbus montanoi* is a doubtful form known only from the type from the Agusan River drainage, eastern Mindanao. *Barbus quinquemaculatus* from the Zamboanga Peninsula is probably a geographical subspecies of *B. binotatus*. *Barbus cataractae* (see Fowler, 1941: 797), also from the Zamboanga Peninsula, is probably a localized variant of *B. binotatus*. After examining the evidence, I suspect that there are really only three well-established cyprinid species in Mindanao outside the Lanao Plateau, one *Rasbora,* one *Nematabramis,* and one *Barbus,* each possibly represented on the island by several subspecies.

Nature of the Lanao Fish Fauna

Barbus binotatus is the commonest, most widespread, and probably the most variable cyprinid of Sundaland (see Weber and de Beaufort, 1916). It ranges from Siam to Singapore, and throughout Sumatra, Java, and Borneo. It is one of the three cyprinids that have been able to cross Wallace's Line at Lombok Strait; the others are forms of *Rasbora* (Brittan, 1954). *Barbus binotatus* exists in most or all of the lowland streams of Mindanao. It exhibits innumerable local races throughout its range.

With no other large endemic lake fish fauna is it possible with such certainty to identify the ancestral species. Lake Lanao was clearly formed rather rapidly, by volcanic action. The ill defined races of *Barbus binotatus* surrounding the Lanao Plateau form the only local source of invasions. Multiple invasions by dissimilar species of *Barbus* or other cyprinid genera are ruled out, unless one wishes to postulate a series of aerial invasions from Borneo, which dropped fishes only on the Lanao Plateau without colonizing the remainder of Mindanao!

Nor are any cyprinids known from Borneo or elsewhere which parallel or are similar to the strange Lanao genera *Mandibularca* and *Spratellicypris*. The same may be true of the genera *Cephalakompsus* and *Ospatulus,* but the types and only known specimens of these two genera were destroyed in Manila.

We are thus forced to the conclusion that *Barbus binotatus* alone gave rise to at least 18 species on the Lanao Plateau, including four new genera. All of the species that I have examined give evidence of derivation from *Barbus binotatus* or at least a close relative. Two or three of the species are only slightly differentiated from *binotatus* and occur both in the lake and its tributary streams. The most distinctive species are known only from the lake itself. *Mandibularca* occurs only in highly turbulent water at the outlet. One or two of the species are said by local fishermen to inhabit only the deeper waters of the lake, while others are found only in the shallow *Potamogeton* beds. *Barbus binotatus* is not known to occur on the plateau, nor are any of the lake species known from below María Cristina Falls, 65 meters in

height, in the Agus River which drains the lake.

The Supralimital Specializations

In 1936, in connection with a report on fishes from Lake Tanganyika, I briefly pointed out (perhaps for the first time) some of the general features of fish evolution in large lakes throughout the world—the African lakes, Titicaca, Baikal, and Lanao. Brooks (1950) has reviewed the subject of speciation in ancient lakes, including Lanao, but Lanao is not an ancient lake, geologically speaking, and the particular features I wish again to stress are neither limited to ancient lakes nor recognized by Brooks.

In Lake Lanao, the peculiar but quite different lower jaw modifications evolved in the genera *Mandibularca* and *Spratelli-cypris* (and probably in *Ospatulus* as well) are approached nowhere else in the very large family Cyprinidae, which is generally distributed throughout Eurasia, Africa, and North America, and exhibits many remarkable specializations. In other words, the jaw modifications of some Lanao cyprinids transcend the familial limits of all the 1,500 to 2,000 non-Lanao cyprinid species in the world. For want of a better term I am calling these supra-limital specializations.

That peculiar supralimital specializations are not confined to Lanao, but are a common and general feature of the evolution of endemic fish faunas in large lakes, is easily demonstrated. The remarkable scaleless cyprinid *Sawbwa* of the Inlé Lake in Burma (Annandale, 1918), the highly modified species of *Orestias* in Titicaca, the extraordinarily modified cichlid genera of Nyasa and Tanganyika, and many of the cottoids of Lake Baikal, all transcend, in one way or another (often strongly and in many characteristics) the limits of specialization of the large, widespread, and varied families to which they belong.

One illustration will suffice. The Percomorphi form the largest order of bony fishes, containing nine thousand species or more. Within the order, many families are defined by relatively few characteristics, of which dentition is often of considerable importance. The fresh-water percomorphs of the family Cichlidae form a large family of perhaps 700 species, distributed throughout Africa, Syria, Madagascar, southern India, and tropical America. Their dental characteristics are generally rather uniform, the modifications usually of small degree. Yet in some of the endemic cichlid genera of Lake Tanganyika, the dental modifications (especially the great, double pointed, heavy-based teeth of *Perissodus* and the utterly strange leaf-like teeth of *Plecodus*) far transcend the limits of dental modification not only of the family Cichlidae, but also of the order Percomorphi and of the entire class of bony fishes. Nothing remotely like them exists. Nor are dental characters the only ones involved. Specializations of the pelvic fins for bottom living (genera *Asprotilapia, Enantiopus*), which elsewhere are considered to be taxonomically of great importance, occur. Indeed, some of the Nyasa and especially the Tanganyika cichlids have come to resemble closely such diverse percomorph families as the Blenniidae (*Telmatochromis*), Girellidae (*Tropheus*), and certain European Percidae (*Asprotilapia*), representing a radiative divergence, and convergence towards different families, entirely unknown elsewhere in the entire gigantic order Percomorphi.

Both *Perissodus* and *Plecodus,* as well as certain other African lake cichlids, might easily be held to represent monotypic families, as has indeed been done with the Comephoridae and (by some) the Cottocomephoridae of Lake Baikal. The late Dr. David Starr Jordan, when shown the jaw of the Lanao genus *Mandibularca,* remarked that a family might well be set up for this genus alone. While I cannot quite agree with this opinion, Jordan's remark is indicative of the situation.

It may be noted that supralimital specializations in fishes are not confined to lake faunas. Any specialization peculiar to one species or genus is, in a sense, a supralimital specialization. However, the general or perhaps the invariable occurrence of extreme and unique specializations in the fishes of lakes that have existed long enough to have produced considerable endemic fish faunas, is notable. Still more notable is the fact that species possessing striking supralimital specializations form a much higher percentage of older lake faunas than they do of stream faunas in general.

The reason for this seems obvious. Most fresh-water fishes inhabit streams and are adapted to life in running water. When lakes are formed, only species already adapted to the slow moving, quiet backwaters are able to take immediate and full advantage of an extensive still-water environment. This extensive new environment usually provides many biotypes not represented in streams, and, in addition, geographical barriers (especially in larger lakes) which may either be present originally or develop with the evolution of the lake itself. The inability of biologists, who are terrestrial animals, to envision these subaquatic facts has greatly hindered studies of fish evolution in lakes.

Stages of Lake Fish Evolution

It is possible to point out sequential steps in the evolution of lake fish faunas, using different existing large lakes as examples; it seems worthwhile to do so. I have specifically refrained from any attempt to evaluate the probably numerous instances in which a relatively small or recent lake has obviously permitted the evolution of one or a few species, sometimes of diverse groups. One such lake is Lake Waccamaw in North Carolina (Hubbs and Raney, 1946). Another is Bear Lake, on the Utah-Idaho boundary, in which three distinct coregonids have evolved (Snyder, 1919). The coregonids have been especially prone to apparent endemism in northern glacial and alpine lakes, but doubt as to the real distinctiveness of many such forms in Postglacial lakes has often been expressed.

In the North American Great Lakes, which have become generally available to fishes only since the geologically recent retreat of glaciation, the coregonids of the "lake herring" (*Leucichthys*) type have experienced a burst of evolution, but many of the endemic species and races are still difficult to separate (Koelz, 1929), if indeed they are really distinct. The fauna is still too young to show anything very definite in the way of supralimital specializations, but the development of species flocks of coregonids is evident. Except for the "lake herrings," no other group of fishes so well preadapted to very cold, still water was present, and this one gained ascendancy.

A similar situation, but probably of greater age because of the greater distinctiveness of the species, is seen in the athernids (*Chirostoma*) of Lake Chapala and other lakes in Mexico (Regan, 1906–1908; Jordan and Hubbs, 1919; Alvarez, 1950) and the cichlids (Meek, 1907; Regan, 1906–1908) of Lakes Nicaragua and Managua. Supralimital specializations among the Cyprinodontidae are clearly foreshadowed in the dwarf, deep-bodied species of *Orestias* in Lake Titicaca (Tchernavin, 1944), which are unlike any of the non-Titicaca *Orestias*.

A clearly more advanced stage is represented by Lake Lanao, in which a single ancestral species of cyprinid has given rise to a species flock, five members of which have become so distinct as to be referable to four endemic genera. Their supralimital specializations have been mentioned above. The excellent work of Mr. Greenwood on the Cichlidae of Lake Victoria shows that the Victoria cichlids are in a state more or less comparable to that of the Lanao cyprinids, although evolution is proceeding on a far grander scale. The species flocks are much larger

and there are four distinctive endemic genera (Greenwood, 1956, 1959), but the ancestral types are either lost or unidentifiable. However, as in Lanao, endemics of families other than the dominant one are absent.

A much older stage is represented by the fishes of Lake Nyasa, which Brooks (1950: 135) estimates to be approximately 500,000 years old. Fryer (1959: 264) gives evidence pointing to greater age. As in all other large Central African lakes, the cichlids (Trewavas, 1935; Fryer, 1959) are dominant. They present the greatest of all known species flocks among lake fishes—over 100 species of the widespread genus *Haplochromis*. In addition, there are over 70 cichlid species belonging to 20 endemic genera, several of which exhibit remarkable supralimital specializations. However, fishes of other families have entered the lake and established endemic species, but only one endemic genus (Worthington, 1933; Jackson, 1959). Most large lakes are drained by physiographic evolution before they attain any age such as that of Nyasa, and it alone remains to represent the evolutionary stage of its fish fauna. The same is true of the two still older lake fish faunas, those of Tanganyika and Baikal.

Lake Tanganyika is at least 1,500,000 years old and may be even older (Brooks, 1950: 148). Its fish fauna (Poll, 1946; 1953) indicates a much later evolutionary stage than that of Nyasa, this being especially notable because of the comparable size and geographical proximity of these two immense Rift Valley lakes. The cichlids are still dominant; they are fewer in number of species than in Nyasa, but the vast majority belong to endemic Tanganyika genera. The only group that could be called a "species flock" is formed by the 19 species of *Lamprologus*, a genus also represented in the Congo.[3] Several of the endemic genera, as has already been noted, are morphologically worthy of familial or subfamilial groupings, and several have come to resemble quite different families of Percomorphi. In non-cichlid fishes, Tanganyika has had time to develop, in addition to a number of endemic species belonging to non-endemic genera, two endemic genera of Clupeidae, two of Bagridae, two of Clariidae, one of Cyprinodontidae (representing a distinctive subfamily; Myers, 1936) and one (*Luciolates*) of Centropomidae (Worthington and Ricardo, 1937; Poll, 1953). Evolution of some of these must have been accomplished in the face of strong competition by the entrenched Cichlidae.

Lake Baikal is the oldest of all, perhaps as much as 75,000,000 years old; its southern basin is Paleocene or possibly even late Cretaceous in age. However, the present lake basin was enlarged and deepened as late as the Pleistocene (Brooks, 1950: 33), and it is doubtful that even the most distinctive Baikal fishes arose prior to the Mid-tertiary. The Cottidae and their derivatives are dominant in Baikal; species of no other fish families are endemic to the lake (Taliev, 1955). The absence of non-cottoid endemics is notable; it is probably due to the poverty of the Siberian fish fauna. The 26 endemic cottoid species belong to nine endemic genera, eight referred by Taliev to two endemic subfamilies of the Cottidae and one genus with two species to the endemic family Comephoridae.

Other lake fish faunas might be fitted into the sequence, but this seems unnecessary.[4]

In all the larger endemic lake-fish faunas, from the youngest to the very

[3] The interesting possibility presents itself that Lamprologus is an autochthonous Tanganyika genus which has colonized the Congo basin.

[4] Some other lakes, with the families to which the dominant endemics belong, are: Lake Biwa, Japan (Cyprinidae); the Celebes lakes (Atherinidae, usually); various Mexican lakes (Atherinidae); the African lakes George, Albert, etc. (Cichlidae); various Central Asiatic lakes, such as Lop Nor, Koko Nor, etc. (Cyprinidae or Cobitidae); Utah Lake (Catostomidae).

oldest, a single family group, preadapted over other stream fishes for lake life, has gained dominance over all others and has retained it. This accounts for my former belief (Myers, 1936) that access to lakes dominated by a single fish family must have been restricted. Access was restricted in Lake Lanao, but probably this has only rarely been true in other lakes. Moreover, in all except the youngest lake fish faunas, supralimitally specialized forms are evident and continue to become more striking until some of them, in the older lakes, could be or are accepted by taxonomists as distinct families.

One other important point should be made. The greater richness in genera and species of the older lake fish faunas, insofar as the dominant family is concerned, compared to the fluviatile fauna of the same family in the same region, is always striking. The Lanao cyprinid fauna dwarfs the cyprinid fauna of Mindanao outside the lake. More than half the African species and far more than half the African genera of the large family Cichlidae are endemics in the lakes of East Central Africa. The greater part of the North American forms of *Leucichthys* are lake endemics. Probably the same is true of Mexican atherinids of the genus *Chirostoma*. The forms of *Orestias* in Lake Titicaca are more numerous than those in the rest of the Andean Altiplano. The cottoid genera of Baikal comprise over three-fourths of the known genera of fresh-water cottoids in the world.

Isolated Endemics

Whether the strange little mastacembelid-like *Chaudhuria caudata* (Annandale, 1918) of the Inlé Lake, sole representative of the family Chaudhuriidae, and the possibly even stranger *Indostomus paradoxus* (Prashad and Mukerji, 1929) of the Indawygi Lake, sole representative of the family Indostomidae, are to be considered as vastly modified relicts of autochthonous lake-fish families, is unknown. If so, they would be the ultimate examples of lake fish specialization, but neither species has any known close relatives, and both may be mere survivors of once widely distributed families. The two genera and three known species of the strange family Adrianichthyidae, from Lake Posso and Lake Lindu in Celebes (see Weber and de Beaufort, 1922), which are undoubtedly derivatives of the family Cyprinodontidae, likewise have no known close relatives by which to judge their exact origin. I would suspect them to be derivatives of the subfamily Oryziatinae, members of which are still widely distributed in fresh waters from India and Japan to Timor, and which have given rise, in India, to the remarkable fish *Horaichthys*. Isolated lake-fish endemics are not too rare, often in lakes in which fishes of another family have become dominant, but the endemic nature of the genus or higher category represented by them is sometimes in doubt. The cottid *Triglopsis* in the American Great Lakes is an example. Perhaps some of these isolated endemics are relicts of previous cycles of lake-fish evolution in the same basins, cycles which were terminated by great changes in the basin itself.

New Areas, New Groups

What has happened, in the normal course of evolution, when one or more representative of an animal group not hitherto represented in the fauna has suddenly gained access to a large area replete with numerous available and unoccupied biotopes, seems to be clear. If the invaders are unable to withstand the competition of the older fauna, they disappear. If they can overcome competition, or especially if there is little or none, rapid or tachytelic evolution occurs, evolution that was impossible in their old home, where better balanced ecological conditions and a balanced fauna held evolutionary divergence more tightly in check. New genera, often utterly unlike their ancestors in one or more strik-

ing characteristics, appear with great rapidity. The rapid proliferation of proboscideans, and their development of supralimital specializations after their invasion of America, is a case in point.

The same sort of evolution has happened time and again when island groups were colonized. The supralimital bill specializations of the Galápagos finches, and (whatever their ancestors may have been) especially those of the drepanidid birds in Hawaii, are well known instances. Island evolution of this kind, like lake-fish evolution, is often striking, because the original colonizers found abundant biotopes totally unoccupied when they arrived.

However, the situation differs somewhat in lakes. The colonizers and founders of evolutionary dynasties in lakes must contend not only with the same types of problems that confront island or continent colonizers. In addition, they must face the change from a flowing to a still-water environment, and, in many instances, problems of depth, pressure, and salinity, perhaps new or inimical to them. In fact, it seems possible that gradually increasing salinity in a closed lake basin might eventually check the evolution of some fresh-water fish groups very severely (Myers, 1938; 1949).

The tachytelic evolution of lake fishes, in part at least representing quantum evolution in Simpson's sense, seems to point out in a really striking way how genera, families, or even higher categories of different animal groups have evolved. If they could get out of their lakes and use their supralimital specializations in other lakes or in streams, as some undoubtedly have done in the past, many existing lake fishes could easily become the founders of large and flourishing new groups at new adaptive levels. Terrestrial groups are not usually as limited in their ability to escape their ranges as are lake animals. As Simpson has so ably pointed out, the tachytelic evolution of new superior groups has seldom left a fossil record because of the speed with which events progressed, and, we may add, because of the probable localization of those events.

It seems probable that events of the sort I have been discussing account for the almost unbelievably rich fauna of characid fishes of the greatest of all rivers, the Amazon. In its present form the Amazon is not an old river. In its lower course it is probably a reversed river; its old structural basin plunges westward. Its Peruvian reaches formed a great lake in relatively recent geological times, and the immense but fluctuating lakes that now line its lower course comprise one of the largest areas of ponded fresh water now existing on earth.

Finally, we cannot forbear to mention the largest of all bodies of still, quiet water, the deep seas. The supralimital specializations exhibited by the highly modified deep-sea descendants of invaders from more turbulent shallow waters have long been the wonder of all zoologists.

It follows that opportunity—the absence of well-adapted competing groups—is extremely important as a factor in the evolution of higher categories. The importance of such a conclusion in relation to the early, rapid evolution of the main animal phyla is obvious.

Summary

1. The endemic fish fauna of Lake Lanao, all belonging to the family Cyprinidae, consisting of a species flock of 13 species and five species referred to four endemic genera, has evolved in a relatively short time, possibly as little as 10,000 years.

2. The distributional facts permit the identification, beyond reasonable doubt, of the single, still-existing, ancestral species that gave rise to the entire endemic fish fauna.

3. Certain specializations of the endemic Lanao genera are paralleled or approached by no others in the large,

widespread family Cyprinidae; because they transcend the morphological limits of all non-Lanao cyprinids, these are termed supralimital specializations.

4. Supralimital specializations are shown to be very characteristic if not invariable features of all large, older, endemic lake-fish faunas; some are so distinctive as to provide characters worthy of family rank.

5. The stages of endemic lake-fish evolution are illustrated by examples, the youngest being the American Great Lakes, the oldest Lake Baikal.

6. A single preadapted fish family represented in the surrounding fluviatile fish fauna assumes dominance in the evolution of large endemic lake fish faunas.

7. The evolution of lake-fish faunas is compared to that of island faunas, and to the evolution of any groups newly admitted to extensive areas where competition is light or absent, and shown to be essentially similar in the relatively rapid production of supralimitally specialized forms.

8. The latter are often capable of becoming the founders of new genera, families, or perhaps even higher categories, at new adaptive levels. They have unquestionably already done so in the older lake-fish faunas, where certain endemic Tanganyika and Baikal genera are worthy of subfamilial or familial rank.

9. It is suggested that the origin of the excessively rich characid fauna of the Amazon River, and of the striking forms and groups of deep-sea fishes, has been due to similar tachytelic or quantum evolution.

10. It follows that opportunity for rapid radiative evolution is of very great importance in the evolution of higher categories, and that such opportunity still may occur from time to time through geological changes.

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ALLOCHRONIC SPECIATION IN FIELD CRICKETS, AND A NEW SPECIES, *ACHETA VELETIS*

RICHARD D. ALEXANDER AND ROBERT S. BIGELOW

Museum of Zoology and Department of Zoology
The University of Michigan, Ann Arbor

and

Department of Entomology and Plant Pathology
MacDonald College, Quebec

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McNeill (1889) was apparently the first investigator to recognize that the most abundant and widely distributed field cricket in northeastern North America is actually composed of two populations, one overwintering as a late instar nymph and maturing in spring and one overwintering in the egg stage and maturing in middle or late summer. McNeill also noted (1) that the adult males of the nymph-overwintering population more often occupy burrows and are characteristically more solitary and more aggressive than those of the egg-overwintering population, and (2) that the ovipositors of the females in the nymph-overwintering population are usually shorter in relation to the length of the body than those of the females in the egg-overwintering population. Later investigators, such as Blatchley (1903, 1920), Walker (1904), Criddle (1925), Urquhart (1941), Cantrall (1943), Fulton (1952), Alexander (1957), and Bigelow (1958) have corroborated and refined McNeill's observations on these two populations without materially altering his conclusions. It is surprising that in spite of the confusion in field cricket taxonomy, the relationships of this pair of populations have been fairly well understood by field biologists for about seventy years.

Prior to Fulton's work, various names had been applied to these two forms, either as binomials or as trinomials. Fulton, Alexander, and Bigelow did not separate the two populations with formal nomenclature. Fulton remained quite uncertain as to their status, being able to deal only with the southernmost fragments of their ranges in the northwestern part of North Carolina. Alexander, puzzled by the apparent identity of the two populations in song, habitat, and distribution, noted that most females could be separated on the basis of ovipositor length, and stated (p. 592), "These two broods may interbreed in mid-summer, or possibly in fall in the southern part of their range, or it may be that they have been isolated such a short time that no noticeable differences have yet appeared between them. Certainly more investigation is needed to clarify their relationship." Bigelow, on the basis of differences he had discovered in the developmental rates of the two populations, and the differences in diapause stage, stated (p. 147), "The distinctive differences between these two populations are more likely to become further consolidated than they are to break down through any future gene exchange. Therefore, these two populations should be regarded as distinct species, however similar they might be morphologically."

Recently, we have pooled our information on these populations and have concluded that a more detailed discussion of their relationships, and their recognition as distinct species, is in order. We believe that these species have become reproductively isolated through a seasonal separation of adults initially imposed by

elimination of all but two widely separated overwintering stages in the ancestral population; there is no evidence that spatial isolation has ever existed, and no necessity for its postulation. The term "allochronic speciation" is used because it seems appropriate to include all speciation resulting initially from temporal separation (cyclic as well as linear) under "allochronic speciation" and all speciation resulting initially from spatial separation (micro-geographic as well as macro-geographic) under "allopatric speciation." "Sympatric speciation" seems to us an inappropriate label because it refers only to spatial relationships which play no more role in the process suggested here than synchrony plays in allopatric speciation.

Acheta pennsylvanicus (Burmeister)
The Northern Fall Field Cricket

The name *Acheta pennsylvanicus* (Burmeister) has most often been used for the nymph-overwintering species, apparently because Scudder (1862) used it for a cricket which he distinguished primarily on the basis of a short ovipositor. As Blatchley (1903, p. 438) pointed out, none of the early American workers gave evidence of having seen Burmeister's types, and the original description (only a sentence) included no measurements. Examination of the types shows that the name was actually applied by Burmeister to the much more frequently collected egg-overwintering species. Measurements of types and examination of descriptions also show that *Gryllus luctuosus* Serville (1839), *G. abbreviatus* Serville (1839), *Acheta nigra* Harris (1841), *Gryllus angustus* Scudder (1862), *G. neglectus* Scudder (1862), and *G. arenaceous* Blatchley (1903) were all applied to the egg-overwintering species (cf. fig. 2 and Alexander, 1957), and are thus synonyms of *A. pennsylvanicus* (Burmeister). This is not surprising in view of the confusion among early taxonomists with regard to this group, and the much greater abundance of fall adults in collections. As shown in figure 2, a single female in Scudder's series of *neglectus* cotypes falls just inside the range of variation of the nymph-overwintering species in the ratio of ovipositor length to body length. Because there is no date on the label, and because this specimen cannot be assigned with any degree of confidence to the nymph-overwintering species, another specimen from this series which is obviously from the egg-overwintering species is here selected as lectotype (fig. 2) to avoid the possibility of name changes at some later date.

Acheta veletis, n. sp.
The Northern Spring Field Cricket

We designate the nymph-overwintering species as *Acheta veletis* in reference to its aggressive behavior. This species can be distinguished from *A. pennsylvanicus* by life history, ovipositor, and behavioral differences, and from other North American field crickets by song, distribution, morphology, and other characteristics (Alexander, 1957).

Holotype male: Collected by Alexander in Piatt County, Illinois, Sangamon Township, 17 June 1954, under a stone in a bluegrass pasture. Head, pronotum, and abdomen black; tegmina and cerci dark brown; hind femora nearly black with a reddish patch extending along the basal one-third of the outer ventral margin; other appendages dark brown or black; pronotum slightly wider anteriorly; head full and rounded, nearly as wide as widest part of pronotum; tegmina reaching to end of abdomen; hind wings reaching to basal margin of third abdominal segment anterior to base of cerci, and completely hidden by tegmina in resting position. Other distinguishing morphological characteristics are not known, except for body measurements given below and shown in figures 2–5.

Allotype female: Collected with holo-

type; same as holotype except legs a little lighter and more reddish in color, and hind wings reaching just past basal margin of fourth abdominal segment anterior to base of cerci; ovipositor a fairly uniform dark brown.

Measurements of holotype and allotype (in millimeters, made with ocular micrometer in binocular microscope): body length, ♂, ♀, 22.0; pronotal length, ♂, 4.3, ♀, 4.5; greatest pronotal width, ♂, 6.9, ♀, 6.7; head width, ♂, 6.9, ♀, 6.4; length of hind femur, ♂, 11.9, ♀, 13.0; length of tegmina, ♂, 13.0, ♀, 12.8; length of hind wings, ♂, 9.0, ♀, 9.5; length of ovipostor, 14.3.

The types are located in the University of Michigan Museum of Zoology. Variations in morphology and in the calling song are discussed by Alexander (1957) under the name, *Acheta pennsylvanica*[1] (Burmeister), spring brood." The different sounds of the males are described and illustrated by Alexander (1957a) under the name "mountain cricket," and appear on a record (Alexander, in press). The calling song consists of 3- to 5-pulse chirps delivered at 120–370 per minute at 85° F. The pulse rate (wingstroke rate) at this temperature is about 25 per second. Aggressive sounds are made by increasing the chirp length, and the courtship song consists of multi-pulse, noise-like phrases delivered at about four per second with a sharp "tick" terminating each prase. There appear to be no differences between the sounds of *veletis* and those of *pennsylvanicus*, suggesting that the breeding populations of the two species have never been in contact on a large scale. No other sympatric species of Orthoptera with identical acoustical behavior are known to us.

There is no evidence of habitat separation of these species; where one is found, the other is also present, and often individual *pennsylvanicus* males are located in the same spots that *veletis* males occupied earlier in the season (Alexander, 1957; Bigelow, 1958). The only known difference in geographic distribution is that *pennsylvanicus* extends into Nova Scotia, while *veletis* apparently does not (Piers, 1918; V. R. Vickery, personal communication). These two species occur over most of the northeastern North America from southern Canada to Maryland and Virginia, down the Appalachian Mountains into western North Carolina and northern Georgia, and west across northern Alabama, Tennessee, and northern Arkansas. The western limits are not known, though they seem likely to angle northwest from about the Oklahoma-Arkansas line following the general line of the Missouri River. Both species may extend to Washington and Oregon, but we have no biological information or song records to confirm the identity of preserved specimens from the northwestern states.

Evidence of Reproductive Isolation

Morphology and behavior. The chief deterrent to recognition of *veletis* and *pennsylvanicus* as distinct species has been the absence of non-overlapping morphological differences, coupled with a lack of information as to reproductive barriers between *pennsylvanicus* and late surviving or occasional second-generation *veletis*. Nearly every worker has noted the slight overlap of adults (fig. 1), apparent because there are no nights during mid-summer when a few singing males cannot be heard.

Morphological comparisons become confusing whenever specimens from all over the geographic ranges of the species are lumped together for the evaluation of individual characters. Both species are quite variable in most of the characters we have studied, but except for a general decrease in size northward, there is little

[1] It has since been pointed out to Alexander by Dr. A. B. Gurney (in correspondence) that *Acheta* is a Greek word, masculine in gender, and the feminine endings used in 1957 for *A. firmus* (Scudder) and *A. pennsylvanicus* (Burmeister) were erroneous.

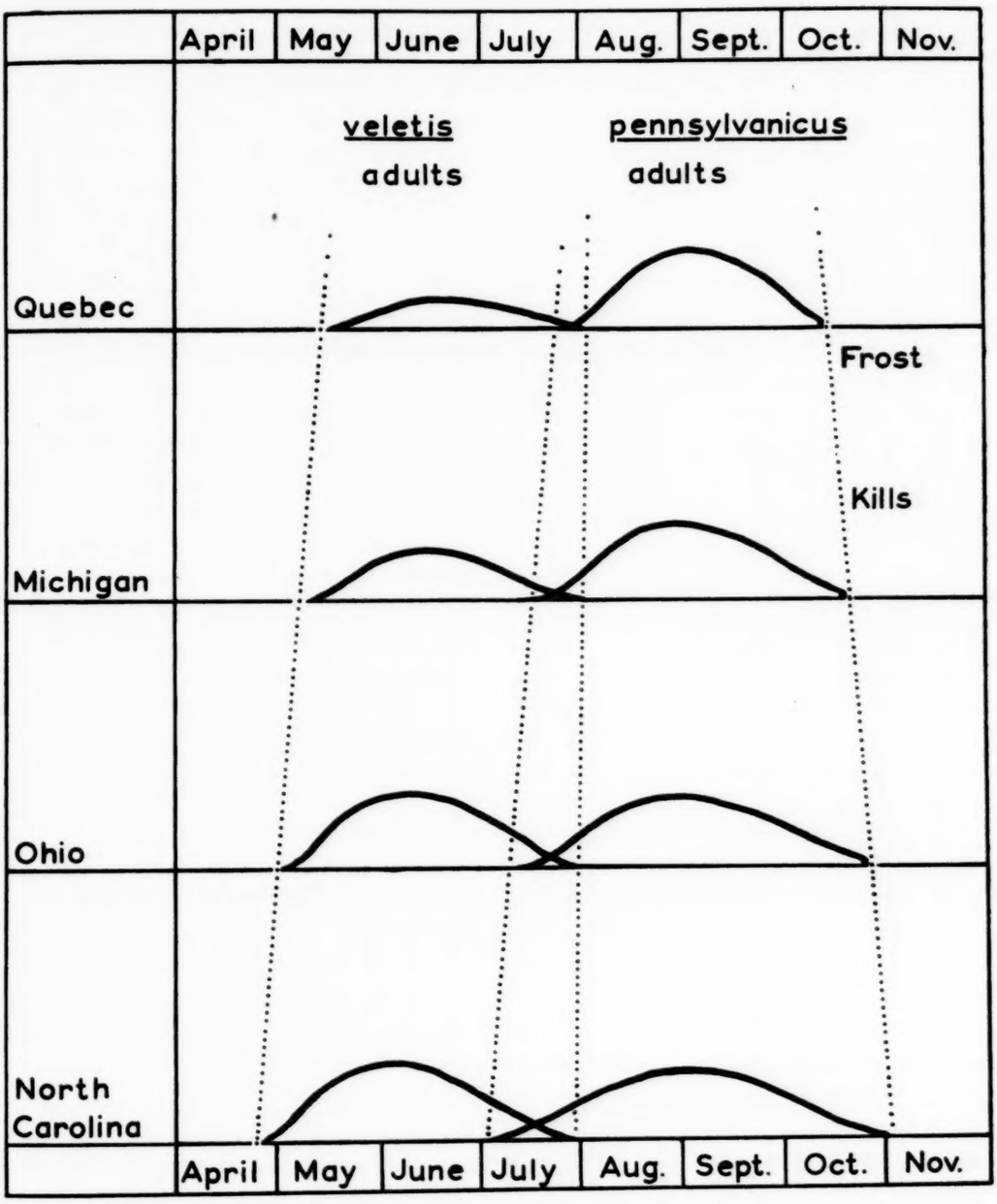
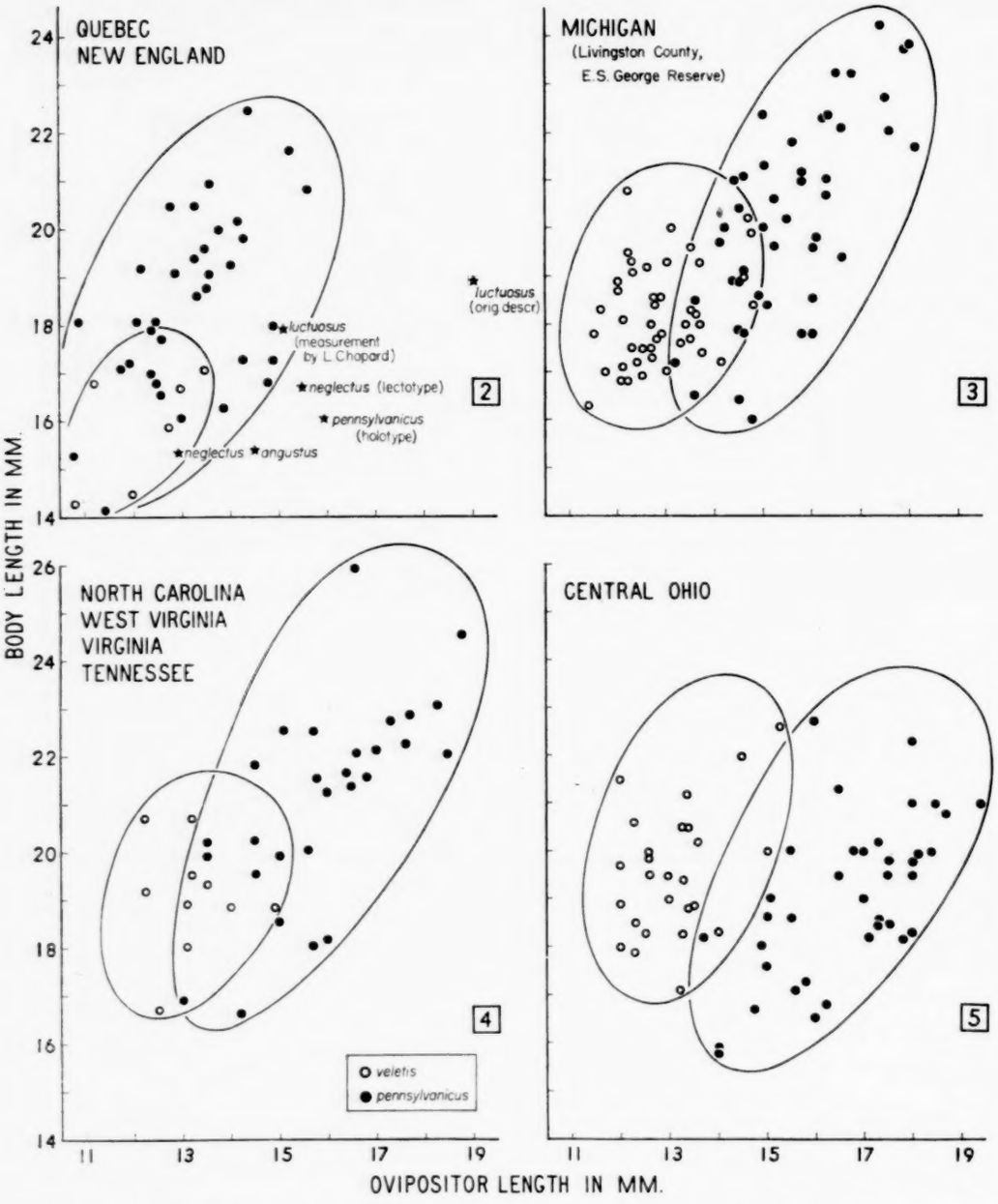


FIG. 1. A generalized diagram showing changes on a north-south plane in the relative sizes of adult populations of *Acheta veletis* and *A. pennsylvanicus*, and in the amount and time of overlap of adults of the two species.

indication of consistent geographic or clinal variation. Specimens from sandy areas (such as beaches around the Great Lakes and along the Atlantic Coast, and inland locations along the Illinois River and in the Kankakee Basin in Illinois and Indiana) have consistently longer ovipositors and are lighter-colored than specimens from other areas. Local populations are often distinctive; for example, a *veletis* population with unusually pale tegmina occurs at Fort Hill in Jackson County, Ohio, and Appalachian populations of either species are apt to be uniformly large and almost entirely black. Occasional Canadian and New England specimens are even smaller than *A. vernalis* (Blatchley), although they retain



FIGS. 2–5. The relationship of ovipositor length to body length in females of *Acheta veletis* and *A. pennsylvanicus* from various locations within their ranges.

the characteristic body conformation of *veletis* and *pennsylvanicus*.

Figures 2–5 show the relationship of body length to ovipositor length in females of the two species from the four areas considered in figure 1. The species were separated on the basis of collection date, and all specimens available from each of the four areas involved were used, with the exception of the few taken in July which could have been either species. Although there is overlap in each scatter-diagram, a high percentage of specimens can be separated, except on the northern border of the ranges.

Selection for longer ovipositors in *pennsylvanicus* has probably occurred because this species overwinters in the egg stage. It has also occurred in both species in sandy areas, and one might

expect *pennsylvanicus* females to have longer ovipositors in the north than in the south. This is not the case; the *pennsylvanicus* females from Canada and New England have ovipositors that are shorter in relation to body length than those in any other population we have studied. Possibly the more consistent cold weather during winter in northern localities is less dangerous to overwintering eggs than the fluctuating temperatures in southern localities.

The ease with which body length is distorted by mounting techniques, and the difficulty in measuring it accurately, led us to search for other characters which would enhance the difference in ovipositor length. However, all those tested (such as femoral length, ovipositor width, and width and length of the head and pronotum) either were valueless or tended to obscure rather than to enhance the difference in ovipositor length. Since there is no reason to assume other than random error in measurement of body length, we believe it is satisfactory for the use to which we have put it. The specimens in figure 5 were pinned and dried, all in the same manner, those in figure 3 were preserved in alcohol, and both kinds of specimens were used in figures 2 and 4.

As mentioned earlier, several workers have noted a general difference between these species in gregariousness and aggressiveness, especially of the adult males, and in the tendency of older nymphs and adults to burrow or to occupy niches and crevices of various sorts. *A. veletis* has thus been described as a sedentary, solitary, aggressive, burrow-inhabiting cricket, while *pennsylvanicus* has been described as a gregarious cricket often found in groups and less often observed in burrows or displaying aggressive behavior. These behavioral differences appear to be fairly consistent in the field, at least in northern latitudes, and this has led to the supposition that they are genetic in origin. However, while differences in these characteristics are apparent among different species of *Acheta*, they can also be produced to some degree between individuals of the same species by varying the situations in which the crickets are reared (Alexander, in prep.). Isolation increases aggressiveness and encourages burrow construction and territorial behavior, and observable differences in these respects between field-collected Michigan males of the two species have been erased in the laboratory by subsequent isolation of *pennsylvanicus* and crowding of *veletis*. Nymphs of *veletis* spend several months in the last three instars, when aggression and burrowing are just beginning to be evident, and are likely to disperse and become isolated. Nymphs of *pennsylvanicus*, on the other hand, mature directly from egg to adult in a few months, and also they do not pass through the periods of cool weather which might be partly responsible for *veletis* nymphs more often occupying crevices and beginning the process leading to solitary, aggressive behavior. Finally, *pennsylvanicus* nymphs are generally more abundant than *veletis* nymphs, and it is significant that the above behavioral differences have been emphasized exclusively by workers in northern latitudes where the difference in population size is most evident. At this point, we must conclude that there is no clear evidence of genetically based behavioral differences of any sort between these two species.

Diapause. Under laboratory conditions, diapause in *pennsylvanicus* and *veletis* is expressed by extensive delay in development in the egg stage and in the late nymphal instars, respectively. At 80–85° F., non-diapause field cricket eggs usually hatch within three weeks; the eggs of *pennsylvanicus* rarely hatch in less than 60 days, and several months may be required. Under the same conditions, non-diapause nymphs usually reach maturity within two months after hatching; in *veletis* and other nymph-diapausing species, a small percentage matures without apparent delay, but most require several months.

The diapause difference between *veletis*

and *pennsylvanicus* has persisted in spite of temperature, season, and light-dark ratio fluctuations in successive generations of laboratory-reared strains at MacDonald College. All of the many thousands of *veletis* eggs successfully incubated in the laboratory have hatched within 47 days, even at temperatures as low as 68° F, while less than one per cent of the *pennsylvanicus* eggs have hatched in less than 40 days, even at temperatures as high as $82 \pm 2°$ F. All *veletis* nymphs were greatedly retarded at temperatures below 73° F, and even at 82° F, about 25 per cent were retarded. The last two or three instars persisted in some cases for three to five months.

The diapause differences between these two species appear to be fundamental. They are infallible in tagging individuals as belonging to one species or the other, they are a direct cause of temporal isolation between the species, and they may be even more directly involved in isolation if differences in the eggs and embryos are involved in the physiological incompatability demonstrated below.

Crossing experiments. It is difficult to obtain adults of *veletis* and *pennsylvanicus* at the same time; in order to do so the normal developmental rate of one species or the other must be altered in the laboratory. Both *pennsylvanicus* and *veletis* seem to require a period of low temperature conditions (in the egg and nymphal stages, respectively) if the vigor and fecundity that exist normally in the field are to be maintained. Consequently, laboratory-reared specimens must be used in controls whenever they are used in tests if the results of the crosses are to be conclusive.

The following experiments were carried out by Bigelow at MacDonald College. The adults were left together in each case until death; in the few cases of premature death, the individuals were replaced.

1. Laboratory-reared Quebec *pennsylvanicus* and *veletis*.

Test	Replications	Offspring
1 vel. ♂ × 3 vel. ♀ ♀	2	many
1 penn. ♂ × 3 vel. ♀ ♀	3	none
1 vel. ♂ × 3 penn. ♀ ♀	2	none
1 penn. ♂ × 3 penn. ♀ ♀	2	many

2. Field-collected Quebec *pennsylvanicus* and laboratory-reared Quebec *veletis*.

Test	Replications	Offspring
6 penn. ♂♂ × 12 penn. ♀ ♀	1	many
6 penn. ♂♂ × 12 vel. ♀ ♀	1	none
6 vel. ♂♂ × 12 penn. ♀ ♀	1	none
6 vel. ♂♂ × 12 vel. ♀ ♀	1	many

3. Field-collected *veletis* from Danbury, North Carolina, Culpepper, Virginia, Frederick, Maryland, and Quebec, and laboratory-reared *pennsylvanicus* from Quebec and Dutchess County, New York. Every combination of *veletis* individuals from different localities was tested, and every test produced many offspring; the same was true of *pennsylvanicus* matings involving Quebec × New York individuals. No offspring were produced in the two crosses attempted: 2 penn. ♂ ♂ (NY) × 3 vel. ♀ ♀ (Va) and 3 penn. ♂ ♂ (NY) × 4 vel. ♀ ♀ (Q).

Eggs were laid frequently in the crosses listed above, copulation was observed once between a *pennsylvanicus* female and a *veletis* male, and the spermatheca of a single *veletis* female examined was found to contain *pennsylvanicus* sperm. Clearly, some effective barrier exists; if hybrids are present in the field they must be exceedingly rare, and actual exchange of genes is even less likely.

Allochronic Speciation

In the vast majority of cases, speciation in sexually reproducing animals probably takes place as outlined by Mayr (1942, 1947) and others. Populations become geographically isolated and diverge through differential selection imposed by their respective environments, and additionally through the accumulation of chance differences in mutations and recombination. If these differences happen

to make the two populations physiologically, morphologically, or behaviorally incompatible, or if they merely make exchange of genetic materials sufficiently deleterious prior to the resumption of geographic contact, then the two populations are irrevocably isolated. With resumption of geographic contact, the resultant interaction between such newly formed species should operate to maximize the efficiency of their isolation and enhance the accelerating and re-inforcing factors operative in their continuing divergence.

Basic to this process is the presence of an extrinsic barrier to gene flow between the two populations with at the same time a sufficiently broad avenue of gene flow within each population to allow consolidation of characteristics leading (eventually) to incompatibility. In sexually reproducing animals these requirements largely eliminate (with certain exceptions—cf. Dethier, 1954) the possibility of speciation along the lines of ecological divergence, which is essentially speciation in the absence of an initial extrinsic barrier to gene flow (Mayr, 1947).

One is faced with certain problems in attempting to reconstruct the evolution of *veletis* and *pennsylvanicus* on the basis of previous allopatry. First, there is no evidence that these species have ever been allopatric; indeed, their wide range, their apparent identity in ecological distribution, and the fact that their geographic limits appear to coincide everywhere but in the north tend to support the opposite suggestion—that they have never differed in geographic distribution more than they do today. The fact that only *pennsylvanicus* occurs in Nova Scotia appears to be more likely a result of the difference in overwintering stage than a reflection of difference in distributional history, and this is supported by the north-south change in the relative abundance of the two species (fig. 1), which indicates that *veletis* nymphs are less likely to survive severe winters than *pennsylvanicus* eggs.

If one postulates that *veletis* and *pennsylvanicus* acquired their present incompatability while geographically separated, he is left with the problem of how they developed such widely different overwintering stages without diverging in other ecological respects. The difference in breeding season cannot easily be explained as a result of interaction after hybridization had become disadvantageous, for the identity in all phases of acoustical behavior and the ability to cross-copulate strongly suggest that the breeding populations of the two species have not been in significant contact since their separation. Further, the lack of difference in ecological distribution reduces the likelihood that the difference in breeding season arose in association with other environmental factors favoring the presence of particular stages at particular times.

But extrinsic barriers need not involve geographic or spatial separation. Bigelow (1958) discussed the possibility that *veletis* and *pennsylvanicus* have speciated without geographic separation through a seasonal isolation of breeding populations. All of the information we have been able to gather with respect to the relationships of these species and the pertinent characteristics of related Orthoptera has reinforced this hypothesis. Three relatively simple steps are involved: (1) the repeated elimination during successive winters of all stages but late juveniles and eggs, with a concomitant reduction or elimination of gene flow between the resulting populations, (2) subsequent increases in the winter-hardiness of the two overwintering stages, culminating in the development of obligate diapauses and (3) the eventual appearance of reproductive incompatibility.

The first step in the above sequence is obviously the most important one, and the one which must be clearly understood if this process can be accepted. How can a species be divided into two populations which exchange genes on a very much reduced basis or not at all, simply by a

differential elimination of overwintering stages? How could this sort of separation persist long enough to allow consolidation of intrinsic isolating mechanisms, and in what sorts of climates could such a phenomenon take place?

An animal susceptible to splitting through the kind of allochronic speciation suggested here for *veletis* and *pennsylvanicus* would be required to possess the following characteristics: (1) a consistent developmental rate producing fewer than two generations per year, (2) a narrow breeding season or a short adult life, and (3) a duality in winter-hardiness, with the two stages involved widely separated on the life history cycle. All of the approximately 85 species of native Gryllidae and Tettigoniidae in northeastern North America possess the first two of these characteristics, and the eight species of Gryllinae there possess all three. North of the 38th parallel, none of these 85 species produces more than a single generation per year. The adults live about six weeks, or less than half the available season, and they generally mature together, producing for each species either a spring or a fall adult population. Only two overwintering stages are represented—late juveniles and eggs—and no species overwinters north of about the 38th parallel in more than a single stage, with the exception of the European house cricket, *Acheta domesticus*, living in artificially warmed situations, and the possible exception of the ant nest inhabitant, *Myrmecophila pergandei*. Two cone-headed grasshoppers, *Pyrgocorypha uncinata* and *Neoconocephalus triops*, overwinter after their final molt, but even these "adults" must be considered immature, for sexual behavior does not begin until the following spring, even in adults kept in the laboratory. Seventy species overwinter as eggs and the rest as late juveniles; all of the latter are burrowing crickets except for the two cone-headed grasshoppers, which apparently spend the winter deep in the bases of clumps of thick vegetation, and which reach northward only to the vicinity of the 38th parallel. One southern field cricket, *Acheta firmus*, reaches to about the 38th parallel and overwinters as both eggs and late instar nymphs there, while breeding more or less continuously and overwintering in all stages farther south.

Duality in winter-hardiness is obviously not unusual in Orthoptera, and it would appear that the burrowing behavior which begins in late juvenile crickets is closely associated with the ability to overwinter. Evidently, nymphs are somewhat less winter-hardy than eggs, even after diapause has appeared, for *veletis* is the most northern nymph-overwintering orthopteran in North America; the other two strictly nymph-overwintering field crickets, *A. vernalis* and *A. fultoni*, which incidentally are much less active burrowers than *veletis*, reach only to the vicinity of the 40th parallel.

All of the native Gryllidae and Tettigoniidae in northeastern North America exhibit obligate diapauses in the overwintering stage, completing development only after considerable delay or after passing through a cold period. These diapauses have an important function in addition to their association with winter-hardiness; they synchronize the appearance of the short-lived adults and are thus responsible for producing a definite breeding season which can be relatively short and yet highly efficient. The cone-headed grasshoppers which overwinter as adults and are not triggered into sexual behavior until spring are important examples. It seems a fair assumption that selection for diapause in connection with synchronizing the appearance of adults in climates such as prevail in northeastern North America is at least as intense as selection for diapause in connection with winter-hardiness.

The nymph-overwintering Gryllinae, including species of *Miogryllus* and *Anurogryllus* as well as *Acheta*, almost

inevitably diapause in the second instar from adulthood. The reasons for this rather precise limitation are not clear, but nymphal diapause has certainly appeared more than once in these crickets, suggesting that this particular stage is unusually susceptible to its development. This is important information, for it strengthens our speculation that nymphal diapause must have appeared independently many times across the range of the nymph-overwintering population that gave rise to *veletis*, as well as egg diapause probably many times within the egg-overwintering population that gave rise to *pennsylvanicus*. There is no reason to believe that this could not have happened while a considerable amount of gene flow was still going on between the ancestral populations of *veletis* and *pennsylvanicus;* whenever and wherever diapause appeared, either in *veletis* nymphs or in *pennsylvanicus* eggs, it surely decreased to a great extent the likelihood of subsequent amalgamation of the two populations.

We would hypothesize the evolution of *veletis* and *pennsylvanicus* as follows. The ancestral population probably inhabited an area of mild winters and bred more or less continuously there. Either an increase in the rigorousness of winters or the isolation of a northern segment of this population (or some combination of such events) produced a population in which all stages but late juveniles and eggs were decimated during each winter. The eggs survived initally because they were buried in the soil, and the nymphs survived initially because of their beginning to actively burrow about three instars from adulthood. We might expect that overwintering nymphs would frequently mature before spring under these conditions, but adults which matured late in the fall would be unlikely to survive until spring because of the short adult life and the tendency of the adults to be active in connection with sexual behavior.

A reasonably consistent developmental rate would now produce a population of spring adults and a population of fall adults. There would undoubtedly be a reduction in gene flow between these two populations, and this condition could persist indefinitely under proper climatic conditions in the absence of genetic differences between the two populations.

Under these conditions, the appearance of diapauses increasing winter-resistance in the already resistant stages and synchronizing the appearance of adults in spring and fall would be advantageous to each of the two partially isolated populations. Wherever such diapauses appeared and were retained, they would further reduce gene flow between the two populations, at the least through emphasizing the appearance of adults only in spring and in fall. The gradual consolidation of diapause in the two different overwintering stages could lead only to more stringent isolation, eventually allowing development of the intrinsic isolating mechanisms present today. As noted, the diapauses themselves may involve morphological or physiological characteristics which produce incompatability. Hybridization at any time during the above sequence would probably be disadvantageous, early in the sequence simply because it would tend to occur in the middle of the season and thus to produce individuals that were likely to be in the wrong stages at the onset of winter, and late in the sequence because almost any manner of inheritance of diapause characteristics would likely cause hybrids to be at a disadvantage additional to that accruing from being in the wrong stage at the wrong time.

The beach cricket, *Acheta firmus*, is especially important in this reconstruction, not only because it illustrates how the process could take place, but also because it may actually be involved in this particular case. This species occurs along the Gulf Coast and the Atlantic Coast from Louisiana to Delaware. In

southern Florida, it appears to breed more or less continuously; in the northern parts of its range it becomes an egg-overwintering species producing a single generation per year, but with a few individuals there getting "out of step with the main population" (Fulton, 1952, p. 289), overwintering as juveniles and maturing in May and June. Not only does this species illustrate duality in winter-hardiness without significant nymphal dispause, but should a northern population become isolated in the area where the species now overwinters only as eggs and as late juveniles, then the separation of that northern population into two species according to the process suggested here for *veletis* and *pennsylvanicus* would be very easy to visualize. It is quite possible that this is exactly what did happen in the past, and that *firmus* represents the southern remnant of the ancestral population which also gave rise to *veletis* and *pennsylvanicus*. Today, *firmus* is clearly more closely related to *veletis* and *pennsylvanicus* than any other North American field cricket, showing close similarity in acoustical behavior, morphology, and developmental rate. The chief difference in morphology among these species is that *firmus* females have very long ovipositors, undoubtedly evolved in association with the beach habitat.

Allochronic speciation as outlined above is a simple and reasonable hypothesis, and the only one which satisfactorily explains all facets of the present characteristics and relationships of *veletis* and *pennsylvanicus*. It is doubtful that the possibility of allopatric speciation could ever be completely ruled out, but the evidence suggests that geographic isolation is both unlikely and unnecessary in this particular case.

Discussion

Speciation through initial seasonal or other cyclic isolation is not a new idea, but the actual process by which it might occur has not previously been outlined in detail for a particular group of animals. Emerson (1949) summarized most of the investigations dealing with this possibility, and more recently Ghent and Wallace have hypothesized speciation through a seasonal separation caused by overwintering in both egg and pupa in sawflies (Hymenoptera). The greatest problem has been in visualizing the initial step; in the process as suggested here, no genetic difference is required, either for the initial separation of populations or for the long-time maintenance of their separation.

A great deal of biological information will have to be acquired before the significance of temporal isolation in helping to account for the prolificness of speciation in insects can be determined. A cyclic phenomenon similar to that discussed here occurs in many insects when populations of species in which the life cycle requires more than a year become isolated by maturing on different years. This happens in many cicadas, most notably between sympatric broods of periodical cicadas, and between those species with 17-year and 13-year life cycles. It may be a more common occurrence than is suspected in other insects as well. Gabbutt (1959), for example, found that *Nemobius sylvestris* (Bosc.), a European ground cricket, has a two-year life cycle, each individual diapausing as an egg one year and as a nymph the next year. The only possibility of gene exchange between populations maturing on different years is through stragglers, and in cicadas, in which the presence of adults can easily be detected because of their loud songs, stragglers are known to be extremely unusual. In such cases, temporal isolation can be as effective as complete allopatry.

The characteristics of animals susceptible to allochronic speciation are obviously not restricted to the Orthoptera; rather they are widespread among invertebrates in temperate climates, especially among

metamorphosing insects. Without the hypothesis of allochronic speciation we would continue to be puzzled by the hundreds of cases in which dichotomy in overwintering stages has become established within groups of closely related species. Such dichotomies have surely arisen many times without speciation, either through the chance appearance of greater winter-hardiness in an unusual stage or through selection for the appearance of the breeding population during a particular season. But when two closely related, sympatric species are found to overwinter in stages which are widely separated on the life history cycle, when there is no evidence for earlier allopatry, and when the two species also maintain brief, non-overlapping periods of reproductive maturity without evidence of previous interaction with respect to characters involved in sexual selection, then it would appear that the possibility of allochronic speciation should not be ruled out without careful study.

Summary

Analysis of what has seemed to be a single species of field cricket, abundant and widely distributed in northeastern North America, shows that it is actually composed of two closely related species which are distinguished as follows: (1) they diapause only in the late nymphal instars and in the egg stage, respectively, (2) they are unable to produce viable eggs in controlled crossing experiments in the laboratory, and (3) most females from the same locality can be separated by ovipositor length. Breeding populations of these two species are seasonally isolated because of the difference in overwintering stage and the short adult life of four to eight weeks. Ecologically and geographically the two species have always been found together, with the exception that *Acheta pennsylvanicus* (Burmeister), the egg-overwinterer, occurs in Nova Scotia, while *A. veletis*, n. sp., the nymph-overwinterer, does not. All of the communicative sounds of the two species appear to be identical, indicating that there has been no interaction with respect to characters involved in sexual selection. It is suggested that these species have become isolated through a seasonal separation of breeding populations imposed by a duality in winter-hardiness originally due to the deposition of the eggs below the soil surface and the burrowing of late juveniles. This initial isolation is then believed to have been re-inforced by the appearance of genetically-based diapauses which not only increased winter-hardiness but also aided in synchronizing the appearance of the adults. The term "allochronic speciation" is applied because of the role of temporal isolation; it is suggested that various forms of cyclic isolation may have been important factors in initiating speciation among insects in temperate climates.

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PATTERN OF CHROMOSOME VARIABILITY IN *DROSOPHILA PSEUDOOBSCURA* [1]

ERIC TERZAGHI AND DANIEL KNAPP [2]

Biology Department, University of Oregon, Eugene, Oregon

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It has been noted by several investigators (Dobzhansky 1944; Novitski 1946; Pavan 1946) that in some species of Drosophila, variability in chromosome structure is virtually restricted to one chromosome. At the same time, they note, the variable chromosome has a large number of inversions distributed throughout the species.

This paper shall be addressed to the former observation, i.e., the concentration of inversions in one chromosome. One possible explanation is that the third chromosome is intrinsically more subject to breakage and rearrangement. This was tested by Helfer (1941) who irradiated *D. pseudoobscura* and demonstrated that the induced breaks and rearrangements occurred at random throughout the chromosome complement. It is the purpose of this paper to present evidence for an alternative explanation for this non-random variation.

It has been shown by Cooper, Zimmering, and Krivshenko (1955) that female *D. melanogaster*, heterozygous for two unlinked inversions, produce an appreciable proportion of inviable eggs; whereas females heterozygous for only one inversion are fully fertile, or virtually so. To explain this phenomenon, they suggest that in the females heterozygous for two unlinked inversions, there is the possibility of non-homologous pairing in meiosis, with unsymmetrical disjunction, and production of unbalanced gametes. If this situation were to hold for a natural population in which one chromosome had acquired variability, any inversion arising in the other chromosomes of the complement would be subject to an extreme negative selection. Thus, whenever such a newly arisen inversion appeared in a female in combination with a previously established heterozygous inversion in another chromosome, the resultant egg mortality would preferentially diminish the frequency of this new inversion.

The experiments described here were set up to determine the extent of egg mortality in females of *D. pseudoobscura* carrying various combinations of heterozygous inversions, in order to determine whether the extent of such mortality is sufficient to allow the above explanation.

METHODS AND MATERIALS

Females heterozygous for no, one, two, and three inversions, from both natural and artificial sources were tested for egg viability. The females to be tested were kept in isolation for 72 hours after hatching, and then were allowed to remain with males of known genotype, for 48 hours. They were then transferred individually, without males, to laying cages which were placed on petri dishes containing a layer of unyeasted corn-meal-molasses medium, blackened with powdered charcoal to facilitate egg counting. The cages were shifted to fresh petri dishes at twelve hour intervals three times, after which the females were discarded. Forty-eight hours after the removal of the cages from a set of dishes, the eggs were counted on that set, keeping tally of the hatched and unhatched eggs.

A survey of the salivary chromosomes was made of the stocks used, in order to confirm their structural constitution. In

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[2] Undergraduate research participant of the National Science Foundation.

those cases where an inversion was carried in stock in the homozygous condition, as with the Arrowhead stocks, it was crossed to a line with standard sequence and the F_1 larvae checked for the heterozygous inversion configuration.

The stocks used were very kindly supplied by Drs. Th. Dobzhansky, C. Epling, and J. Schultz. They include the following:

I. In (I) Ssc (short scutellum), pericentric inversion of the X chromosome with associated phenotype of shortened scutellum; found by Esther Rudkin Novitski.

II. In (II), medium-short, subterminal, radiation induced inversion of the second chromosome.

III. In (III), Arrowhead, short, subterminal naturally occurring inversion of the third chromosome.

IV. In (IV), medium-short, submedian, radiation induced inversion of the fourth chromosome.

TABLE 1

Genotype		Egg counts	Per cent
Female	Male	Unhatched/total	viability
+	IV	104/2,031	94.9
+	III	35/854	95.9
Average,* no inversion females			95.2
I	+	23/304	92.4
III	+	8/264	97.0
I	III	34/564	94.0
III	III	475/5,489	91.3
III	III; IV	210/4,800	95.6
III	II; III; IV	76/520	85.4
Average,* one inversion females			93.1
I; II	+	30/249	88.0
II; III		330/2,174	84.8
III; IV	III	869/3,028	71.3
II; III	III; IV	165/1,370	88.0
III; IV	II; III	47/164	71.3
II; III	II; III; IV	88/387	77.3
III; IV	II; III; IV	90/574	84.3
Average,* two inversion females			78.8
II; III; IV	III	146/392	62.8
II; III; IV	II; III	215/638	66.3
II; III; IV	III; IV	255/461	44.7
Average,* three inversion females			58.7

* Group averages are weighted.

RESULTS

In table 1 the results of each experiment are reported. The inversions given are all in the heterozygous condition.

The data clearly demonstrate that in *D. pseudoobscura*, as in *D. melanogaster*, egg lethality is correlated with the number of unlinked, heterozygous inversions in the female.

The no and one inversion females have an insignificantly different percent egg viability, with 95.2% and 93.1% averages, respectively. When the egg viability of the no inversion females is adjusted to 100%, the two inversion females produce 82.7% viable eggs and the three inversion females produce 61.7% viable eggs.

DISCUSSION

D. pseudoobscura is restricted to western North America and Central America. The inversion pattern, and its distribution in this region, was first worked out by Dobzhansky and Sturtevant (1938) and expanded by Dobzhansky (1944). From these works it is evident that *D. pseudoobscura* meet the conditions of the hypothesis of this paper. There are a large number of widely distributed rearrangements of the third chromosome; and a very few rearrangements of the other chromosomes, which have a very restricted distribution. Dobzhansky and Sturtevant found twelve common gene sequences, falling into two large phylads, in addition to a number of rarer inversions in the third chromosome. Although inversion frequencies for each strain were not given, the data was given in such a fashion that it was apparent that in some strains, a particular sequence was homozygous, whereas in other strains, two or more sequences were represented.

In the other chromosomes, one com-

plex of inversions is found in the X, "sex ratio," and is widely distributed throughout the range of the species. It should be noted that "sex ratio," by virtue of its expression should be capable of forcing itself upon a population against a considerable selection gradient. That is, a female structurally heterozygous in the third chromosome and heterozygous for "sex ratio" will experience a depressed egg hatchability, but one half of her male offspring will transmit almost exclusively the "sex ratio" inversion complex. Along these same lines, it would be of considerable interest to repeat the experiments of Wallace (1948), varying the structural constitution of the third chromosome in the population cages, and observing the effects on "sex ratio" frequency.

Two different endemic inversions of the second chromosome have been found. One, Santa Lucia (Tan, 1936), is found in one locality only, and the other was collected in three locations adjacent to each other. In both cases, the third chromosome is apparently homozygous for one particular gene sequence. One endemic inversion of the fourth chromosome has been found, in one locality, where the third is heterozygous for several gene sequences. This peripheral variation, in non-third chromosomes, may represent the initiation of variation which will increase in frequency with the passage of time, or it may simply represent several of many abortive "attempts" at introducing non-third chromosome variation. In the two cases of the second chromosome inversions described, if the third chromosome is in fact nearly, or totally, homozygous for one sequence, there would be no selection pressure against the newly arisen second chromosome inversions. Hence, it could conceivably become well established in that particular sub-population. However, the one fourth chromosome inversion described would encounter the adverse selection pressure, because of the structurally heterozygous third chromosome in the same region; and one would expect it to disappear rapidly.

It would provide very interesting relevant information if the populations showing second and fourth chromosome variability were to be sampled again after a period of years. It could then be observed whether the frequencies of these inversions behaved as predicted above; and if not, perhaps some explanation would be evident.

It was mentioned in the introduction, that, in addition to the problem of the concentration of variability in one chromosome, there is the problem of the existence of a large number of different inversions in the variable chromosome. As a solution to the latter problem, Novitski (1946) suggested a mechanism by which a heterozygous inversion can produce new, closely related inversions more readily than totally *de novo* inversions can be produced. As evidence, Novitski pointed out the distinct grouping of breakpoints in the third chromosome rearrangements of *D. pseudoobscura*. More recent work by Goldschmidt (1956) on *D. subobscura*, and by Rothfels and Fairlie (1957) on *Chironemus* provide a considerable bulk of evidence in support of the Novitski hypothesis.

As a final conjecture, it may be suggested that the hypothesis of this paper plays a positive role in preventing gene flow between incipient or closely related species. That is, two species, or diverging sub-populations, may differ by little more than gene sequences in all or most of the chromosomes; but the occasional hybrid would be subjected to such great negative selection, due to its very low relative reproductive potential, that it would simply not be maintained in the population.

The extensions of the hypothesis, here suggested, to other species showing the same structural variability pattern, must be done with caution; because the principles of chromosome-mechanics now

known to apply to *D. melanogaster* and *pseudoobscura* may simply not operate in some of these other species. Each species would require its own experimental tests. Likewise, one should regard cautiously, the converse, namely, if multiple structural heterozygosity leads to reduced egg viability, then this particular structural variability pattern should be observed. There are undoubtedly several factors affecting the pattern and distribution of structural variability, any of which could override the effect described in this paper.

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Summary

A hypothesis is suggested to explain the high degree of variability in respect to inversions found in the third chromosome of *D. pseudoobscura* with a corresponding low degree of variability found in the other chromosomes.

The hypothesis is based upon the observation, in *D. melanogaster*, that females with two or more unlinked, heterozygous inversions, produce fewer viable eggs than females with no or one heterozygous inversions.

The experimental work conducted indicated a considerable reduction in the number of viable eggs produced by a female carrying two or more unlinked heterozygous inversions.

It is considered that this reduction is sufficient to account for strong negative selection against the accumulation of inversions in chromosomes other than the highly variable third in *D. pseudoobscura*.

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HERITABILITY OF GEOGRAPHIC VARIATION IN TRUNK SEGMENTATION IN THE RED-BACKED SALAMANDER, *PLETHODON CINEREUS*

RICHARD HIGHTON

Department of Zoology, University of Maryland

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It was recently shown by Highton and Grobman (1956) that there is geographic variation in the number of trunk vertebrae in two species of salamanders, *Plethodon cinereus* and *P. richmondi*. In the red-backed salamander, X-ray photographs of samples from New Brunswick, Indiana, New Jersey, Virginia, Tennessee and North Carolina indicated that in each area the modal number of trunk vertebrae was 20, while all of the specimens from Georgia possessed more than 20 trunk vertebrae (range, 21 to 23). On the basis of this variation, the Georgia populations were recognized as a separate subspecies, *P. c. polycentratus*.

Highton (1957) outlined a method that made it possible to estimate, with 94% accuracy, the number of trunk vertebrae in *Plethodon* by counting the costal grooves. This method has been used to estimate the number of trunk vertebrae in available museum material from the known range of the red-backed salamander. The resulting data indicate that the amount of geographic variation in number of body segments is greater than indicated in our original report. In addition to the Georgia populations, there are a number of others which also average 21 or more trunk vertebrae. These occur in eastern Long Island, the southern portion of the Del-Mar-Va Peninsula, the southern Coastal Plain of Maryland, and parts of the Piedmont and Coastal Plain Provinces of Virginia and North Carolina.

There is also a region, including the western portions of New York, Pennsylvania and Maryland, adjacent northeastern Ohio, and parts of eastern and central West Virginia, in which the modal number of trunk vertebrae is 19. The usual number of trunk vertebrae is also 19 in a portion of the Catskill Mountains of New York and in southeastern Missouri. The remainder of the extensive range of the red-backed salamander is occupied almost entirely by populations with a modal number of 20 trunk vertebrae. There are a small number of populations which are intermediate in that they possess approximately an equal number of specimens with 19 and 20 trunk vertebra or with 20 and 21 trunk vertebrae. Most of these are from regions that are situated geographically between the areas outlined above. Figure 1 indicates the distribution of red-backed salamander populations with modal counts of 19, 20, and 21 or more trunk vertebrae. Further details of this variation will be presented in a later paper on the systematics of the genus *Plethodon*, which will discuss the taxonomic implications of geographic variation in trunk segmentation as well as other geographically variable characters.

There are a number of ecological differences among the three regions with different numbers of trunk vertebrae, but there is also much variation in the environment within any one of the regions. In light of the evidence from experimental studies on the effects of temperature and other environmental variables on the number of vertebrae in fishes (see Tåning, 1952, for a review of the literature), the validity of the number of trunk vertebrae as a taxonomic character in salamanders may be questioned. If the observed geographic variation is solely phenotypic, due to variations in tempera-

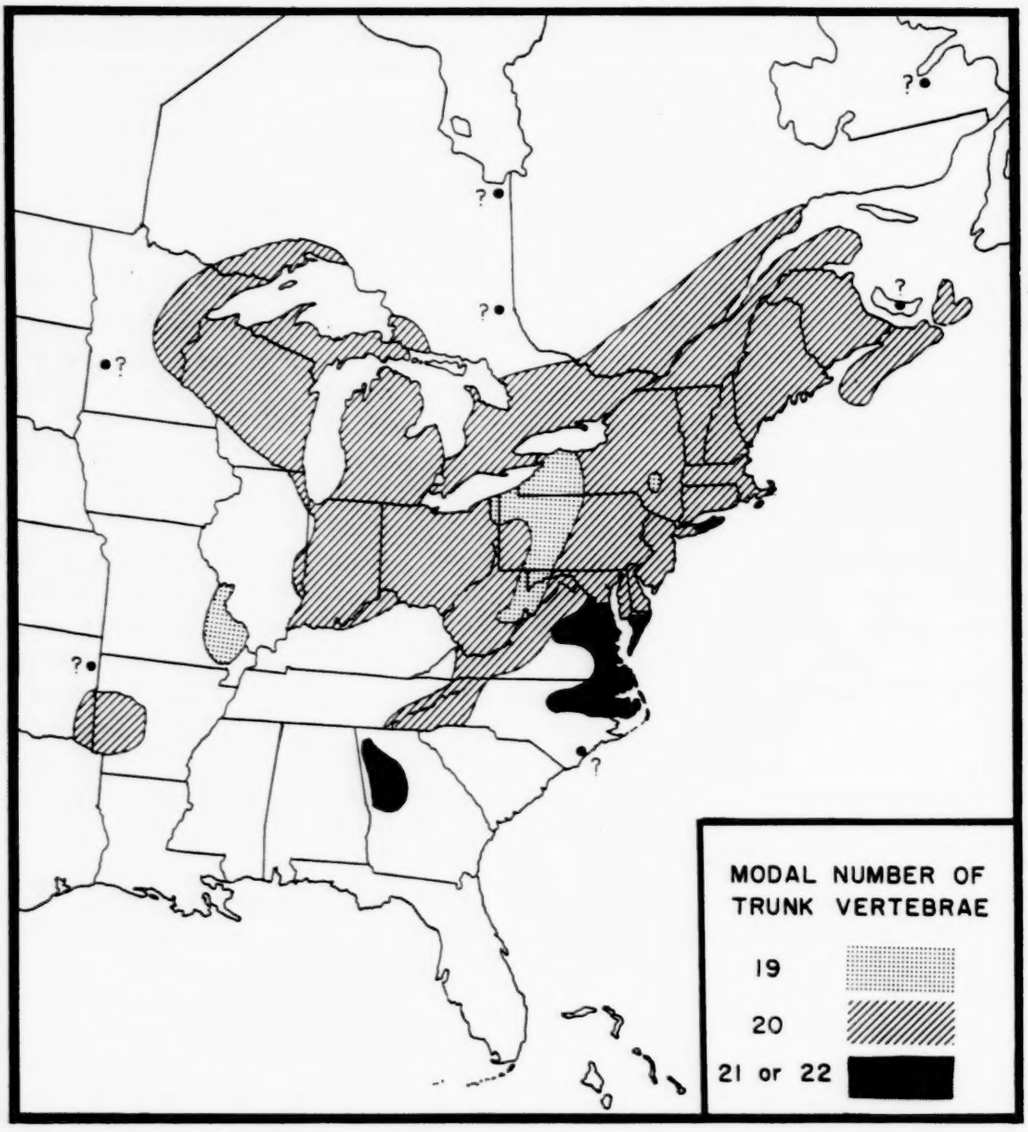

FIG. 1. Geographic variation in the modal number of trunk vertebrae in *P. cinereus*. Two dots represent questionable locality records (Hudson Bay and Labrador), and the others represent localities from which series of specimens are not yet available (for example, there are only two specimens from Prince Edward Island and both have 19 trunk vertebrae).

ture, altitude, and/or some other environmental factors, then no genetic differentiation affecting the number of vertebrae has evolved. On the other hand, if the differences are genetically determined, then the observed variation is due to evolutionary divergence.

Data have been obtained from two sources which provide information relative to the influence of heredity on variation in number of trunk vertebrae. First, a large number of females associated with their broods in nature were collected from a single geographic area. If the variation in number of vertebrae within a population is due to genetic factors, it would be expected that the average number of vertebrae in the offspring of a female would be correlated with her own vertebral number. Secondly, females from a number of populations with different mean vertebral counts have been

induced to deposit eggs in the laboratory. All of the embryos were raised at the same controlled constant temperature. If the differences among the different geographic populations are hereditary in nature, it would be expected that the offspring would have about the same number of vertebrae found in the populations from which their parents were derived.

Table 1 illustrates the type of individual variation that occurs in samples from eight different populations of *Plethodon cinereus*. In most samples of the hundreds that have been examined, there is a variation of no more than three vertebrae, and a majority of the specimens in each population possess the medial number of trunk vertebrae. A small percentage, usually from 2% to 6%, of the specimens in each population have asymmetrical vertebral counts. The most frequent cause of asymmetry is the articulation of the left and right ilia with the sacral ribs of two successive vertebrae. Occasionally, asymmetry is due to the irregular fusion of two or more vertebrae. In calculating the means and variances, the average of the two sides of asymmetrical specimens was used.

TABLE 1. *Geographic and individual variation in trunk segmentation illustrated by selected samples of* P. cinereus *from eight localities*

Locality	Number of trunk vertebrae											Mean	Variance
	18	18/19	19	19/20	20	20/21	21	21/22	22	22/23	23		
Allegany State Park, Cattaraugus Co., N. Y.	31	9	329	5	36	1						19.01	.177
Fort Littleton, Fulton Co., Penna.			78	10	86		1					19.53	.248
Cunningham Falls State Park, Frederick Co., Md.		1	93	12	580	6	59					19.95	.209
Big Meadows, Shenandoah National Park, Va.			20	2	130	3	17		1			20.00	.246
Mt. Bethel, Northampton Co., Penna.	1	1	97	35	928	43	208		1			20.09	.247
1 mi. WNW of College Park, Prince Georges Co., Md.			3		253	14	150		1			20.37	.245
Near Millwood, Clark Co., Va.					40	4	62		2			20.63	.263
2 mi. NE of Palmetto, Fulton Co., Ga.							18	1	25		2	21.64	.318

RESULTS

An estimate of the heritability of trunk segmentation in *P. cinereus* was obtained from 90 broods collected near Mountain Lake, Giles County, Virginia. The spent females accompanying all but 12 of these broods were captured. It is assumed that each female found associated with a nest is the parent of the brood concerned. Altogether, 3,451 specimens (exclusive of offspring, but including the brooding females) were collected from 31 localities within six miles of the town of Mountain Lake. The broods were all collected after July 23, when the embryos were close to hatching, and long after the limbs were developed, so presumably subsequent conditions in the laboratory would have had no effect on their ver-

TABLE 2. *Variation in trunk segmentation among populations of* P. cinereus *in the vicinity of Mountain Lake, Virginia. Distances are from the town of Mountain Lake, Giles County, Virginia*

Locality	No. of broods	Number of trunk vertebrae					Population	
		19	19/20	20	20/21	21	Mean	Variance
4¼ mi. N, 1½–2 mi. E.	37	222	60	1002	20	67	19.87	.209
4¼ mi. N, 1¼ mi. E.	16	58	21	374	9	34	19.94	.197
4¾ mi. N, 2 mi. E.	25	71	21	471	16	41	19.95	.193
Other localities	12	175	37	705	8	39	—	—
Total	90	526	139	2552	53	181	19.89	.206

tebral number. The data on samples from populations in which broods were collected are given in table 2. All but 12 of the broods were taken at three localities, where large samples are available. There are slight differences in average vertebral counts among the samples collected in the vicinity of Mountain Lake, but most of these differences are not statistically significant. Considering the amount of geographic variation that occurs in this species, the variation among localities near Mountain Lake, Virginia, is very slight.

The hatchlings were reared in the laboratory for several weeks until the yolk was resorbed and they had reached a size that was sufficient to estimate the number of vertebrae from their costal groove counts. The data are summarized in table 3. The offspring of females with 19 trunk vertebrae have the lowest average number of body vertebrae, while those from females with 21 vertebrae have a higher average number of trunk vertebrae than offspring of females possessing 20 body vertebrae. Only the phenotype of the female parent is known, since the male parent (or parents) never guards the nest.

Using the method of path analysis (Wright, 1923), it is possible to obtain an estimate of the heritability of trunk segmentation (the fraction of the phenotypic variance due to heredity). The paths between the phenotypes of the female parent and a single offspring as well as the average of her brood are shown in figure 2. The correlation coefficient between the genotype of an individual and its phenotype, h, is the square root of its heritability, h^2. The correlation coefficient between the phenotypes of a parent and a single offspring is $\frac{1}{2}$ h^2 under the assumption of no environmental correlation between parent and offspring; therefore $h^2 = 2\, r_{PDPO}$. Since there is a number of offspring in

TABLE 3. *Summary of trunk segmentation in female parents and their offspring in 90 broods collected in the vicinity of Mountain Lake, Giles Co., Virginia*

Number of trunk vertebrae of female parent	No. of broods	Number of trunk vertebrae of offspring					Mean
		19	19/20	20	20/21	21	
19	7	24	4	17			19.42
19/20	3	12	1	14			19.54
20	60	82	20	253	2	9	19.78
20/21	2			11	1	3	20.23
21	6		2	31	1	1	20.01
Unknown	12	4	1	37	1	3	19.98
Total	90	122	28	363	5	16	19.78

many of the broods, the estimate of heritability would be too high unless the correlation among sibs is taken into account. The sib-sib correlation is calculated by partitioning the variance into its component parts (see table 4). The within population parent-offspring correlation coefficient, $r_{P_D P_{\bar{O}}}$, is equal to .429, and the sib-sib correlation coefficient is equal to .337. The estimate of heritability was found to be .57, based on the 488 offspring from female parents of known phenotype, indicating that over half of the variance in trunk segmentation is due to hereditary factors. It is probable that this estimate is too low because of the coarseness of classification of the phenotypic variation. For example, several different genotypes probably result in a phenotype of 20 trunk vertebrae. All permit the embryo to pass the threshold between 19 and 20 trunk vertebrae during development and some undoubtedly approach the threshold between 20 and 21 vertebrae, but do not reach it. Even if environmental variation had no affect on this threshold (heritability equal to 1), then the inability to detect these different genotypes would result in a lowered estimate of the heritability. Epistatic deviations may also slightly affect the estimate of heritability, as well as errors in the estimates of the vertebral number in the hatchings.

Another method of estimating the her-

TABLE 4. *Analysis of variance of number of trunk vertebrae in the offspring of* P. cinereus *from the vicinity of Mountain Lake, Giles County, Virginia*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	487	110.94	.228
Among populations	8	7.10	.888
Broods within populations	69	42.57	.617
Within broods	410	61.27	.149

Coefficient of among brood variance component, n (average brood size adjusted for variation in brood size among populations)	6.15
Variance within broods	.149
Variance component among families within populations	.076
Correlation between female parent and mean of brood	.429
Correlation among members of same family within populations	.337
Heritability, h^2, based on correlation of means of broods with female parents within populations, adjusted to a single record basis	.57

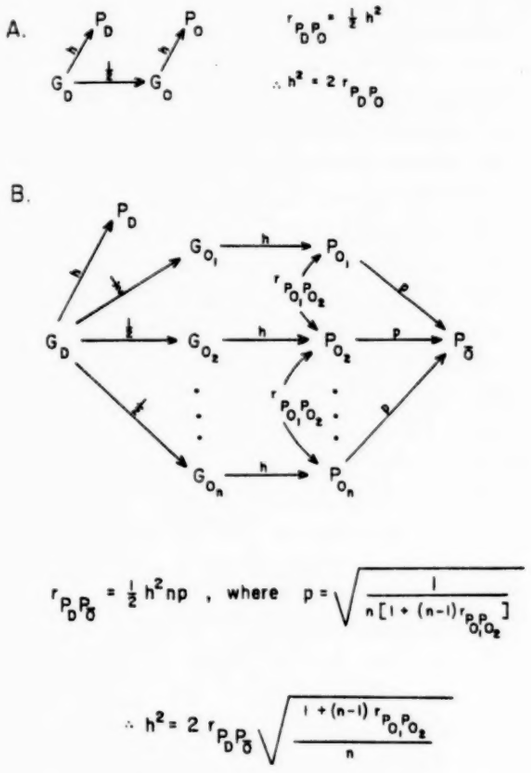

FIG. 2. Heritability calculated by means of path relations between parent and offspring. A. Correlation between phenotypes of parent and single offspring. B. Correlation between phenotypes of parent and mean of brood. G refers to genotype and P refers to phenotype; the subscripts D, O, and Ō refer to the female parent, the offspring and the mean of a brood; h is the correlation coefficient between the genotype of an individual and its phenotype, and h^2 is the heritability; p is the correlation coefficient between the phenotype of a single offspring and the average phenotype of its brood.

itability of trunk segmentation in these parent-offspring data is by the use of the regression coefficient of the average number of vertebrae in the brood (considered the dependent variate) on the number of trunk vertebrae in the female parent (the independent variate). Twice the regression coefficient should provide an estimate of the heritability. This estimate of heritability has certain advantages over that based on the correlation coefficient in that the expected value does not depend on the number of individuals in the brood and it is not biassed by a restriction of range of the independent variate. The calculation of heritability using this alternate method, corrected for differences among populations from which broods were collected, yielded an estimate of heritability of .61. This is very close to the figure obtained by the use of the correlation method, but is probably a better estimate.

In the vertebral estimates of the offspring collected in the Mountain Lake area, there may be a bias in the direction of underestimating the number of vertebrae. The difference between the vertebral counts of the large population samples (3,451 specimens; mean, 19.89) and those of the 534 offspring from the same populations (mean, 19.78) is statistically significant. If this work is ever repeated, it would be especially desirable to raise the young salamanders to an age when their vertebrae are sufficiently well-ossified to be able to obtain an accurate count from X-ray photographs. If the difference in the vertebral counts of the Mountain Lake population samples and the offspring taken from these populations is real, it would be an indication of a differential mortality rate in the post-hatching period. There does not appear to be any significant difference in the number of offspring per brood in the females with different vertebral counts.

The above analysis shows conclusively that a substantial amount of the individual variability within a population is attributable to genetic factors, but provides no information relative to the genetic basis of geographic vertebral variation. In order to evaluate this type of variation, gravid females from thirteen different populations of *P. cinereus* were induced to deposit eggs in the laboratory by injections of mammalian gonadotropic hormone using the method of Anderson (1958). Four of these populations are listed in table 1. All of the females were kept for at least a week at 18° C before they were treated with the hormone. Oviposition took place during the period from April 21 to June 1, 1959, probably an average of about one month before the natural egg-laying season. None of the salamanders deposited eggs during the period of the experiment without hormone treatment. Ovulation therefore almost certainly occurred as a result of the hormone injections, with internal fertilization taking place in the laboratory. Since all of the females had been isolated since their capture, mating must have previously occurred in the field. Therefore both parents of each offspring were derived from a single natural population. In all, 476 eggs were deposited in the laboratory by a total of 123 females. About 70% of the eggs either failed to develop or the embryos did not survive to a stage when it was possible to count the number of trunk vertebrae. There was no apparent differential mortality in the broods from different geographic populations. All eggs were placed on paper toweling saturated with water in small glass jars. All were incubated in the same constant temperature incubator at a temperature of 18° C. Care was taken to avoid exposing the developing embryos to higher temperatures for more than a few seconds while being examined at weekly intervals during development. All broods were treated in the same manner during examination. The induction of egg-laying in specimens from different populations was not randomized with respect to time,

so it is not known whether or not there was any seasonal effect.

The number of trunk vertebrae in the offspring was estimated in two different ways. Some hatchlings were reared in the laboratory until they had reached a sufficient size to count the costal grooves. Others, that failed to survive long enough to make possible an accurate costal groove count, were cleared with potassium hydroxide and stained with alizarin red. The girdles are not ossified at hatching so that it is impossible to determine the sacral vertebra directly by the articulation of the ilium with the sacral rib as in larger specimens. The number of trunk vertebrae in the cleared and stained material was estimated by subtracting four vertebrae from the number which lacked haemapophyses. In this species, the atlas, trunk vertebrae, sacrum and the first two caudal vertebrae usually lack haemal arches. Data for the two different methods of estimating the number of trunk vertebrae are in essential agreement for offspring from the same population, and in addition, both methods were used on 25 specimens with 76% consistency of estimates.

The results, summarized in table 5, clearly show that the number of trunk vertebrae in the offspring is correlated with the number of body vertebrae in the population from which they were

TABLE 5. *Comparison of trunk segmentation of offspring from different geographic populations reared in the laboratory*

Locality	Population		Female parents		Offspring	
	Sample size	Mean no. of vertebrae	No.	Weighted mean no. of vertebrae	No.	Mean no. of vertebrae
Fort Littleton, Fulton Co., Penna.	175	19.53	5	19.43	14	19.43
Cunningham Falls State Park, Frederick Co., Md.	751	19.95	11	19.88	25	19.92
1.1 mi. ESE of Bristol, Anne Arundel Co., Md.	23	20.09	1	21.00	2	20.50
Blair's Valley, Franklin County, Penna.	9	20.11	4	20.08	13	19.81
0.9 mi. S of Huntingtown, Calvert Co., Md.	18	20.17	1	20.00	3	20.00
2½ mi. ESE of College Park, Prince Georges Co., Md.	124	20.22	8	20.48	23	19.87
1 mi. WNW of College Park, Prince Georges Co., Md.	421	20.37	5	20.03	17	20.29
Glenn Dale, Prince Georges Co., Md.	16	20.41	5	20.36	11	20.32
Near Tuckahoe, Cape May Co., N. J.	26	20.42	4	20.43	7	20.79
0.6 mi. SE of Patuxent, Charles Co., Md.	55	20.44	4	20.60	10	19.90
1.5 mi. SE of Glenn Dale, Prince Georges Co., Md.	19	20.61	4	20.31	8	20.25
Near Millwood, Clark Co., Va.	108	20.63	5	20.65	10	20.40
3 mi. N of Upper Marlboro, Prince Georges Co., Md.	33	20.76	1	21.00	1	21.00

TABLE 6. *Analysis of variance of number of trunk vertebrae in laboratory-reared specimens of* P. cinereus *from different geographic populations*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	143	54.04	.378
Among populations	12	16.00	1.333
Broods within populations	45	15.72	.349
Within broods	86	22.32	.260

Coefficient of among brood variance component, n (average brood size adjusted for variation in brood size among populations)	2.35
Variance within broods	.260
Variance component among families within populations	.038
Correlation between female parent and mean of brood	.086
Correlation among members of same family within populations	.128
Heritability, h^2, based on correlation of means of broods with female parents within populations, adjusted to a single record basis	.12

derived, in spite of the fact that all were raised at a constant temperature and in otherwise similar laboratory conditions. The correlation coefficient between the mean of the population and the mean of the offspring from the same population is .74. This is a high correlation, considering the random sampling errors implicit in the small sample sizes of offspring from several of the thirteen populations and the probable errors due to indirect estimate of vertebral number. It is highly unlikely that there is an environmental effect on the sex cells before fertilization takes place, which would influence the number of vertebrae in the developing embryo. Unless this is the case, these data demonstrate a hereditary basis for a considerable amount of the vertebral variation among the geographic populations used in the experiment.

Applying the same methods of analysis that were used to estimate the heritability of the field collected broods, the correlation method gives a heritability estimate of .12 (see table 6), while that for the regression method is .16. The average size of the broods is much smaller, due to the 70% mortality in the laboratory-reared specimens, thus lowering the sib-sib correlation. In the analysis of variance, a great deal of the total variance is among populations. The correlation between the phenotype of the female parent and the phenotype of the average of her brood is also much lower than in the Mountain Lake material, but this is probably mainly due to the small sample size of both number of offspring within broods and numbers of broods within most populations. The number of broods from each population is not sufficient to provide a meaningful estimate of heritability, as in the case of the Mountain Lake data.

DISCUSSION

The experimentally reared embryos were not obtained from extreme populations of *Plethodon cinereus*. The specimens were taken from populations with means differing by no more than 1.2 vertebrae, less than half the known variation found in natural populations (see table 1). It would be desirable to repeat these experiments, using material from more divergent populations.

As yet, there is no information on the specific effects of any environmental variable. Variation in temperature or other ecological factors may prove to be partly responsible for the variation in the number of trunk vertebrae among geographic populations of these salamanders. It is possible that heritability might be different under other environmental conditions, or it may vary geographically. The within-brood variance of the laboratory-reared sample was .26. This is higher than the within-population variance of many natural populations (see table 1), which may indicate that the laboratory

conditions affected the variation in vertebral number. Actually the genetic variance within broods should be less than the genetic variance of the entire population. If all the members of a litter are full sibs, the genetic variance within a brood would be only one-half the genetic variance of the population, whereas if they are half-sibs, the within-brood genetic variance would be three-quarters of the total population genetic variance. There is no information on the sexual behavior of these salamanders, so that it is impossible to know with how many males a single female may mate. In the specimens from the Mountain Lake area, the within-brood variance is only .149, less than the variance of all of the samples from populations in the area (see table 2).

The phenotypic variance of all the Mountain Lake populations combined is .206. If about 61% of that variance is due to heredity (or .126) and the variance within broods is .149 (from table 4), then it could be suggested that most of the families consist of full sibs, since the difference between the two (.057) is about half the hereditary portion of the variance of the population. However, a more constant environment within a sibship may also play a part in reducing the within-brood phenotypic variance, in which case the same result could be obtained even if all the offspring in each brood were half-sibs.

The notion, expressed above, that the heritability of within population vertebral variation may be greater than .61, is further supported by the field collected broods from the Mountain Lake area. Not a single offspring of females with 19 vertebrae possessed more than 20 vertebrae, nor did any of the offspring of females with 21 vertebrae have less than 20 vertebrae (except for two asymmetrical specimens). This would be expected if polygenic inheritance with random mating was operative. Even when a 19 vertebrae female mates with a male with a large number of high-vertebrae genes (with a phenotype of 21 trunk vertebrae), the offspring would be expected to be approximately intermediate between the two extremes and would probably have no more than 20 trunk vertebrae. The converse would be expected in the offspring of females with 21 trunk vertebrae, and none of the 35 offspring of such females has a vertebral count of 19. It might be expected that fewer offspring of females with 21 vertebrae would resemble their female parents than the offspring of females with 19 vertebrae. This is because, in the Mountain Lake populations, there is a higher proportion of specimens with 19 vertebrae (17.2%) than with 21 vertebrae (6.0%). Thus the genes for the lower number of vertebrae would have a higher frequency in these populations, and the mating of two 21-vertebrae individuals would be an infrequent occurrence.

Summary

Geographic and individual variation in the number of body vertebrae in the salamander *Plethodon cinereus* has been described.

The number of trunk vertebrae in 488 offspring in 78 broods collected in the vicinity of Mountain Lake, Giles County, Virginia, was shown to be correlated with the number of body vertebrae in their female parents, showing that part of the variation within a population is due to genetic factors. The estimates of heritability obtained from these data are .57, based on the use of the correlation coefficient, and .61, based on the regression coefficient method.

Females from thirteen different geographic populations were induced to deposit eggs in the laboratory by the injection of mammalian gonadotropic hormone. The number of trunk vertebrae in the 144 offspring reared in the laboratory under constant controlled temperature conditions resembled the vertebral

counts of their parental populations, thus suggesting a genetic basis for at least a part of the geographic variation in trunk segmentation.

Acknowledgments

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THE EVOLUTION OF THERMIPHILOUS WATER MITES[1]

RODGER MITCHELL

Department of Biology, University of Florida, Gainesville, Florida

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The animals found in hot springs are commonly tolerant forms from adjacent waters and the few known obligate thermophiles are, as a rule, locally restricted species. Water mites of the genus *Thermacarus* are a prominent exception. Both described species are obligate thermophiles (requiring temperatures always over 32° C), yet they are widely distributed; one occurs on either flank of the Himalayan Mountains, the other is known from 21 widely separated springs in the western United States. Another water mite genus of the new world, *Partnuniella*, also contains widely distributed obligate thermophiles.

The problem considered here is how to explain the distribution and thermophily of these and other water mites. The direction taken by any consideration of the problem is a function of whether one assumes that thermophily evolved first and was followed by an extension of range or that some widely distributed form had a tendency to acquire thermophily independently in many locations. The first line of argument requires proof of host transport over long distances between springs or else the existence of a nearly continuous series of hot springs in eastern Asia, between Asia and North America, and in western North America and neither suggestion seems likely. The second approach must show that the mites of some widespread habitat are predisposed to the occupation of, and persistent reproduction in thermal waters.

The adaptations for locomotion on wet shores involve profound modifications of exoskeleton and musculature and the two unrelated mites that are most extreme in these modifications are both widely distributed and facultative thermophiles. It is clear that this combination of features is perfectly consistent because these animals locomote in a habitat where a very wide temperature tolerance is essential.

The discussion will begin with a consideration of the two unrelated facultative thermophiles, *Trichothyas* and *Tyrrellia*, in order to demonstrate that the parallel adaptations in these animals are related to thermophily. Then speculations on the evolution of such adaptations can be used in the explanations of thermophily among the obligate thermophiles, *Thermacarus* and *Partnuniella*.

THE ANATOMY OF WATER MARGIN INHABITANTS

Mites with extreme adaptations to water margins thrash their legs helplessly in deep water because some force greater than gravity must press the mite to the substrate in order for the legs to gain traction. This force is a result of the surface tension of the water drawn over the mite by its hydrophilic integument. Therefore, these mites are restricted to wet surfaces covered by a film of water not as deep as the mite is thick. Such forms can burrow in addition to crawling but none of the known species are characteristically burrowers.

The adaptations of the genus *Trichothyas* to water margins have been described elsewhere (Mitchell, 1957). Exoskeletal features are used in the discussion below, but the musculature has

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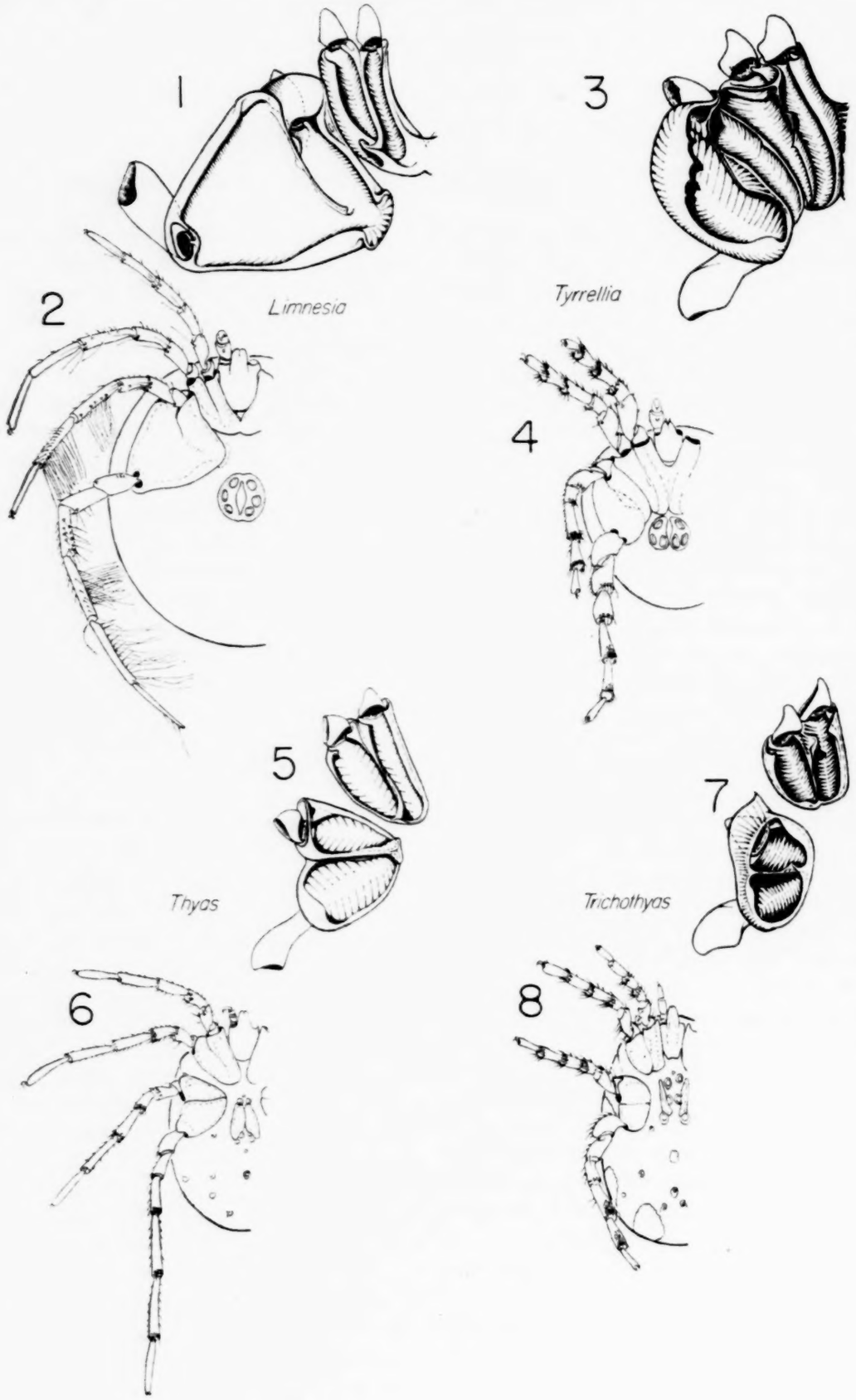
1
2
Limnesia
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also been briefly studied to verify the interpretations. Virtually all the features discussed below are illustrated in figures 1–8.

The legs of the water margin inhabitants, *Tyrrellia* and *Trichothyas*, (figs. 4, 8) are modified as follows: 1) a progressive shortening and widening of all leg segments, 2) a great expansion of tips of all the segments that contact the substrate, and 3) development of heavy broad setae on the ventral surface of most segments.

Legs with these modifications tend to dig into the substrate so that forces developed by the leg muscles move the body rather than the legs. The short segments of considerable girth house large muscles which develop greater force because of the wide separations of muscle insertions from the segmental articulations. Both the expanded ends of the segments and their terminal setae dig into the substrate so as to anchor the legs and this is further augmented by the setae along the venter of the leg segments.

The maximum volume and length attained by the muscles acting upon basal segments of the legs can be estimated from the size and shape of the coxae. Three features of coxal anatomy are peculiar to the crawling forms of water margins: 1) the low length/width ratio of the coxae (length being taken along the long axis of the coxae which extends parallel, or nearly so, to the axis of the extended leg), 2) very high and heavy apodemes around the margins of the coxae, 3) rotation of the articulations for legs I and II anteriorly, and 4) widening of the end of the coxae to accommodate the broad basal leg segment.

High coxal apodemes provide great lateral areas for muscle origins and, of course, only short muscles can lie within the foreshortened coxae. These short thick muscles insert on the basal leg segment which is of such a large diameter as to greatly separate the muscle insertions from the points of articulation. All these features contribute to the power of leg action. Because of the rotation of articulations, the plane of basal segment movement in legs I and II is changed from an approximately vertical plane to a horizontal plane, consequently, both the segmental flexors of the legs and coxal muscles act together.

Tyrrellia and *Trichothyas* locomote similarly. The film of water drawn over the body by the hydrophilic integument holds the mite down to the substrate. When legs III and IV are elevated and flexed and the basal segment rotated so as to depress the leg, the expanded tips of the segments anchor in the substrate. The leg must extend as it is depressed and therefore pushes the body forward. Legs III and IV act similarly in other water mites and only the surface tension keeps the mites in water margins from being elevated off the substrate. Normally legs I and II lift the body and through the action of the segmental flexors the animal is pulled toward the anchored leg tips. But the force generated by segmental flexors is apparently not effective under water margin conditions. When the coxal articulations are rotated anteriorly legs I and II move in a horizontal plane and muscles originating on the coxae can retract the leg base at the same time as segmental muscles flex the leg. Thus both muscle groups can act in concert in water margin forms instead of acting independently as is the case in other water mites. In deeper water the mite rests lightly on the substrate and legs I and II fail to dig in; legs III and IV tip and

FIGS. 1–8. The general body form of two water margin inhabitants (*Tyrrellia* and *Trichothyas*) in comparison with that of their unspecialized relatives (*Limnesia* and *Thyas*). Figures 1, 3, 5, and 7 show the inner aspect of the coxae which serves as a gauge of the length and breadth of the muscles acting upon the legs and Figures 2, 4, 6, and 8 give the general form and relative leg size as viewed from the ventral aspect.

roll the mite about, therefore, the mite is unable to make controlled movements in open water.

These two animals must be pressed to the substrate, but less modified mites can locomote in both open water and water margins. Thus, moderate water margin adaptations do not necessarily preclude occupation of other habitats, but every mite of water margins does have some of these structural adaptations. The conditions necessary for the operation of these locomotor devices are an adequate habitat description: water margin habitats are situations along the edge of a body of water where there is an undisturbed film of water over the substrate that is no deeper than the thickness of the body of the mite. This tautologous definition is the only suitable way to discriminate the habitat of the mites. Waves disrupt water margins and only the shores of smaller water bodies and streams, spring seepages, and the splash zones of water falls provide extensive stable water margin habitats.

The Anatomy of Thermacarus

These mites show some but not all of the adaptations of highly modified crawlers. The legs (fig. 12) are much too heavy and short to be used in swimming and the setae on the parts of legs one through three that contact the substrate are modified as in *Trichothyas* and *Tyrrellia*. Internally the coxae are found to be relatively short and broad with extremely high lateral walls (fig. 13) which indicates the presence of short heavy muscles. Finally the articulations of legs I and II are rotated so that they move in a more nearly horizontal plane.

Field observations suggest that the leg action of *Thermacarus* is comparable to that of the water margin forms considered above. But the previous examples were obligate water margin inhabitants whereas *Thermacarus* is a faculative water margin inhabitant. The long legs provide a broad base supporting the body and the large claws provide a secure footing so *Thermacarus* can easily walk in still water.

Temperatures Experienced by Water Margin Forms

Any undisturbed film of water is heated by insolation and cooled by evaporation so it is rarely at the same temperature as either the air or the main water body. Measuring local temperature fluctuations on surfaces is complicated by the problem of measuring temperature in a thin film and it is only recently that thermisters or thermocouples with small sensing elements have been readily available. Such equipment is essential for recording surface film temperatures as Tuxen (1944) pointed out. To discover the temperatures experienced by the mites one must record the temperature of the wet surface at the exact location of each observed mite. Such a procedure has not been followed in the past so there is no acceptable published data on the temperatures water margin inhabitants experience.

All the 1958 and 1959 temperature records were read from a metal encased thermister element 10 mm in diameter and 2 mm thick. This could be placed flat on the substrate and would stabilize within 10 seconds at a reading that was not significantly altered by either shading or exposure to sun during the operations. The readings were quite consistent under field conditions and are thought to be accurate within one or two degrees Centigrade.

Temperature data for *Tyrrellia* are plotted in figure 9 which illustrates the extremes of temperature around the margin of a small pond in southern Michigan (Crane Pond on the E. S. George Reserve of the University of Michigan near Pickney, Michigan), where *Tyrrellia ovalis* Marsh. and *Tyrrellia circularis* Köen. are common. Records were taken by maximum-minimum thermometers with bulbs rest-

ing on the water margin substrate where *Tyrrellia* occurred and in the water just off shore. Daily records were retained only from periods when the water levels were stable. Five daily records were obtained and the extremes of temperatures are indicated by horizontal lines in figure 9. The maximum range of temperature at a depth of 30–45 cm in the center of the pond is given by the vertical lines and it shows the range of temperature in water margins to be at least twice that recorded in open water. The high temperatures last no more than six hours and are followed by a rapid cooling to temperatures as low as or lower than the adjacent water body.

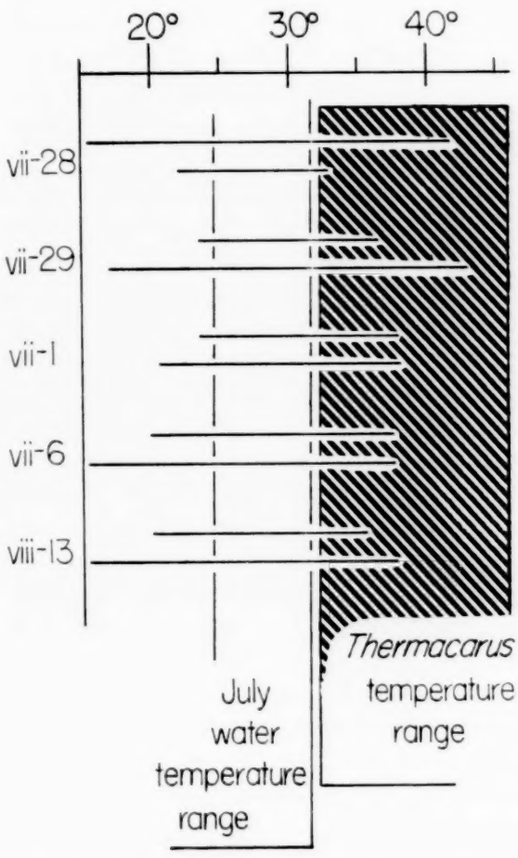

FIG. 9. The range of temperatures (in degrees Centigrade) experienced by *Tyrrellia* species along water margins are indicated by the horizontal bars. The temperature of the central area of the pond and the temperatures experienced by an obligate thermophile are indicated. (Procedural details are explained in the text.)

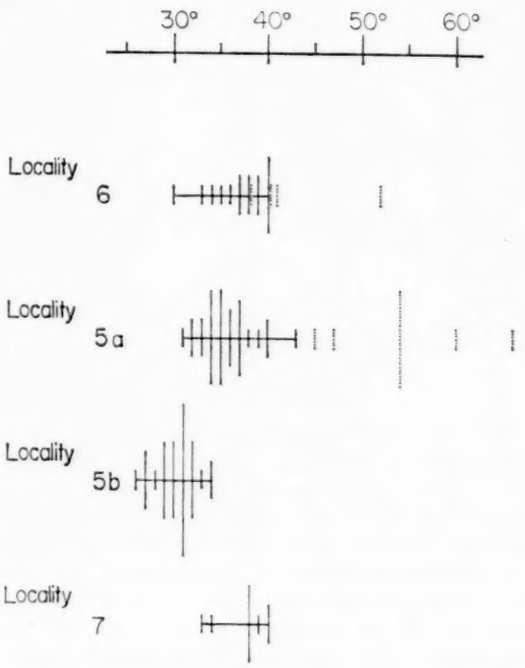

FIG. 10. Temperatures (in degrees Centigrade) at which *Tyrrellia cf. circularis* were found in water margins (Locality 5b) as compared with the species when collected in hot springs (Localities 5a, 6, and 7). Vertical bars indicate the frequency of live individuals at a temperature. Dotted lines indicate dead specimens. Sample sizes were: Locality 5a, 36 mites; Locality 5b, 28 mites; Locality 6, 19 mites; Locality 7, 9 mites. (Procedural details are explained in the text.)

The temperatures tolerated by *Thermacarus* (indicated by the shaded area of figure 9) shows that *Tyrrellia* is exposed to temperatures as high as those experienced by a thermophile.

The data in figure 10 are from a situation (locality 5) where a species of *Tyrrellia* (*T. cf. circularis*) occurs both along a river shore and around adjacent hot spring flows. The springs must be inundated at times and only mites that move up the banks can survive so the populations cannot be isolated. Temperatures were taken at the point of capture of each specimen and two midday samples—one around the hot springs (5a) and one on the river shore (5b)—were recorded. The temperature overlap in these samples is so great that no differentiation of habitats can be made.

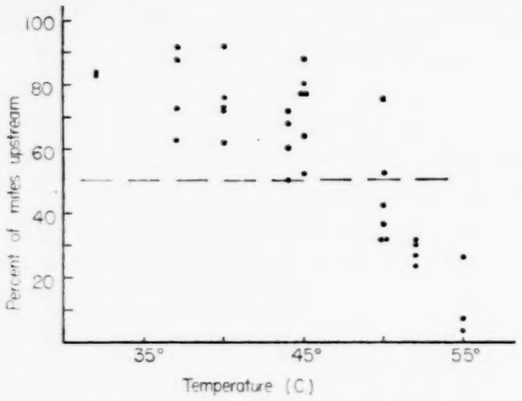

FIG. 11. The responses of *Thermacarus nevadensis* to current as a function of temperature. The points on the graph are based on samples of 25 or 30 specimens. Each of five different samples were tested in both ascending and descending series of temperatures and the data were perfectly consistent regardless of the sequence of temperatures or the sex of the specimens. The experimental procedure is explained in the next.

Occupation of hot springs is hazardous for nine of the 36 specimens (25%) taken at locality 5b and four of the 19 specimens (21%) taken at locality 6 were dead. Dead specimens were collected at higher temperatures (45° to 60° C) than any living specimens and it seems unlikely that the dead specimens represented several days accumulation for the mites were intact and held together in handling. Evidently *Tyrrellia* cannot avoid zones of lethal temperature.

These data also show that non-thermal water margins may be defined only on the duration of high temperature and when classified on this basis there is an array of habitats from water margins that reach high temperatures for a short time during summer days to springs of perpetual high temperatures. *Tyrrellia* occupies all these habitats (if temperatures are less than 41°–43° C) and there is obviously no biological response that will justify separation of thermal from non-thermal water margins in this instance.

Tyrrellia has been found in six hot springs: *T. circularis monensis* Mars. from a spring on Paoha Island, Mono Lake, California (Marshall, 1940); *T. cf. circularis* Köen from localities 5, 6 and 7; and *T. cf. ovalis* Mars. from localities 1 and 4 (numbered localities are listed in the Appendix). Until the mosaic of variation within and/or between *Tyrrellia* populations is clarified the use of species names will be uncertain. Two complexes of variable populations resembling either *T. circularis* or *T. ovalis* are known to occur in water margins over most of the United States.

The second water margin genus, *Trichothyas*, is found around cool springs or spring fed streams and has been considered as a cool water form, an association that makes their occasional occupation of hot springs enigmatic. They are often in waterfall splash zones which are not necessarily cool. One *Trichothyas* species is not stenothermal and two other species are in very warm waters. Indirect evidence suggests that the other species of the genus are, in reality, eurythermal and so there is room for doubt as to the significance of the "cool" spring association. "Cool" springs can be refugia for either warm weather or cold weather relicts (Nielson, 1950; Mitchell and Mitchell, 1958) and it could be that *Trichothyas* species of temperate regions can live only where the low winter temperatures are ameliorated.

Zoogeographic data supports this suggestion. The southerly species are found in mountainous tropical regions with species described from Java, Central America, and Central Africa. Northern species are found around the Mediterranean, on Madeira, in England, and in Jugoslavia; in northern India and Japan; and in central and southeastern United States. *Trichothyas* species are either not present or extraordinarily local in central or northern Europe or in the northern tier of the United States. These distribution data show that *Trichothyas* species are in warm or at least moderate climatic zones.

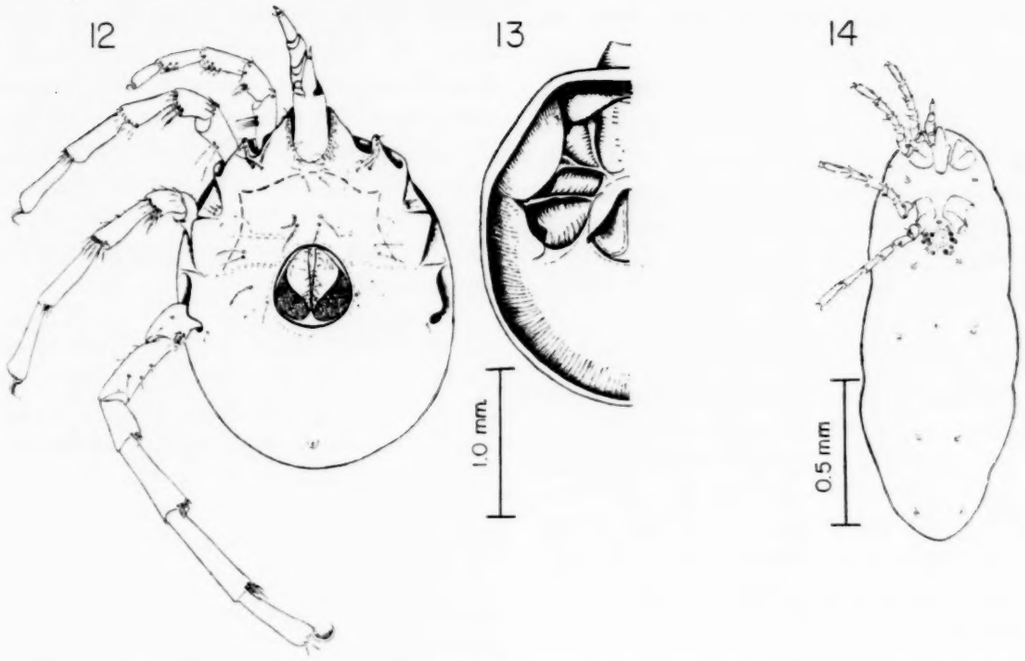

FIG. 12. Ventral aspect of a female *Thermacarus nevadensis* with the appendages of one side drawn in.
FIG. 13. Internal aspect of the venter of a female *Thermacarus nevadensis*.
FIG. 14. Ventral aspect of *Partnuniella paucipora*.

The two facultative thermophiles, *T. feuerborni* Viets (1935) and *T. japonica* Uchida and Imamura (1953) are not necessarily associated with cool waters. *T. feuerborni* is known from around waters of 9° C to 36.3° C and *T. japonica* from a spring of 40° C. Although these temperatures of the main water body are not those experienced by the mites there is no doubt that constantly warm waters are suitable habitats. All the data on locomotor habits and distribution indicates that *Trichothyas* species may tolerate temperatures approaching 30–35° C around a "cool" spring margin.

T. muscicola Mitchell (1953), an inhabitant of "cool" spring seepage zones is known to experience temperatures as high as 34° C (table 1). At the time these data were recorded the mite had little choice of temperature for most of the surface was at 29°–30° C with only limited zones as low as 25° C or as high as 34° C. The temperatures to which the mites were exposed reached the lower limit of thermophilous mites (cf. table 1 and fig. 9). Obviously this *Trichothyas* species has a range of temperature tolerance similar to that of *Tyrrellia* species and these temperature tolerances would appear to be essential to the animals because of their similar locomotor adaptations.

TABLE 1. *Temperatures at which* Trichothyas muscicola *were found to occur at Rock Creek Canyon (west of Kankakee, Illinois) on August 22, 1959. The data were taken at mid-day when the air temperature was around 30° C*

Temperature in degrees Centigrade	24	25	26	27	28	29	30	31	32	33	34
Number of times a mite or a cluster of mites was found	1	—	1	—	1	7	3	3	3	2	1
Total number of mites	2	—	1	—	3	13	3	3	7	13	2

Temperature Zones of Hot Springs

Heat loss is related to the surface area exposure and the capacity of the substrate and air to absorb heat. Cooling is most effective if the water splashes over a terrace or exposed slope and the loss of heat will vary as a function of the air temperature, wind, insolation, and, to a small degree, humidity. Cooling is most constant and slowest in a flow through a distinct channel and is primarily due to loss of heat to the air through the surface. A more constant but minor loss of heat occurs laterally into the substrate and climatic factors hardly affect this heat loss. When these matters are taken into consideration three situations of very different temperature characteristics can be defined.

1. The shallow flow of thermal waters over exposed surfaces. Except near the point of origin all such flows vary maximally in response to climatic factors and usually have a constantly shifting flow pattern as well. Under these conditions an animal is not able to restrict itself to specific temperatures and must tolerate a wide range of temperatures.

2. Within, or immediately in contact with, the flow of a confined stream. Temperature zones are determined by volume of flow, and initial spring temperature and the zones move up or downstream as a function of climatic factors. The movement of isotherms is regular and oriented with the current and variations are in a line with stream flow so that an active animal directed by appropriate taxes could limit itself to a restricted temperature zone.

3. Margins of defined stream flows. Even in the most porous substrate temperatures run from the stream temperature to the normal soil temperature within a centimeter or two. Climatic factors act here but their effect is probably slight and the main variable is stream temperature which is increasingly constant toward the source. Temperature zones of the water film on the wet shore near the source will rarely shift at all but the zones are narrow (the temperature may drop nearly ten degrees in a centimeter). An animal in margins must orient to temperature for slight movements perpendicular to the water body can put an animal in dangerously high temperatures.

Apparently truly thermophilous animals must be associated with the margins of the immediate overflow of a hot spring or else with a defined stream leading from the spring (situations 2 and 3) because they present suitable temperatures and allow an orientation to temperature.

Zones of no less than 30° to 40° C were shown to occur around most nonthermal water bodies at mid-day. Thus, a defined stream carrying off the spring effluent will provide a continuous avenue along which water margin forms of nonthermal waters can invade hot springs. I believe that this explains the independent invasion of hot springs by water-margin forms. There is now the task of interpreting the zoogeography and ecology of the strictly thermophilous mites of the western United States in light of these speculations.

Ecology of Thermacarus nevadensis Marshall

Attempts to delimit the habits and habitat of *Thermacarus* failed because the number of different ecological associations about equalled the number of localities. Admittedly the localities chosen for protracted studies were selected to give a wide range of conditions, but, nevertheless, it was surprising to find that temperature was the only constant related to the occurrence of *Thermacarus*. In the following brief summaries the individual *Thermacarus* populations are reviewed. (Full data on the localities follows in an Appendix.)

Locality 1. Most of the area quickly cools to air temperature in a terraced

flow where *Thermacarus* was not found. Records of specimens were from the vicinity of confined flows and the mean temperature of 20 records was 40.7° C (range 33°–48° C). *Thermacarus* was very difficult to find here and perhaps the fact that there were very restricted zones where temperatures remained within the above range is of basic significance. All mites were burrowing in the substrate where there was a very thin covering water film and they had the habits of typical water margin forms.

Locality 2. *Thermacarus* was never marginal and was taken in bottom samples in still water pools at depths of 10–20 cm. Presumably they burrowed into or walked over the flocculent debris of the bottom but the cloudy water made observations impossible. Three very large pools, two at 37° C and one at 42° C, supported abundant populations.

Locality 4. Here *Thermacarus* burrowed in the substrate in a small pool of 45° C water at the base of a talus slope. Only one mite was taken downstream where the water flooded over a flat area. The area at a suitable temperature was small and the mite was rare.

Locality 6. *Thermacarus* was common both in upstream water margins and downstream among the flooded bases of sedge and grass clumps. Temperature ranges were similar in both situations; the average for 46 records was 44.5° C (range 39°–50° C).

Locality 7. The springs were similar to the previous locality. Very large numbers of *Thermacarus* burrowed and walked over the entire bottom in the stream course where temperatures ranged from 32° C to 40° C. Seventeen collections of *Thermacarus* from margins averaged 40.2° C (range 34°–45° C).

These data refer to adults and nymphs of established populations and do not reflect the random meanderings of a few individuals. The bulk of a given resident population is found in only one kind of situation which seems to be determined by the temperature conditions encountered. Large populations were found on the bottoms of still pools, in areas of moderate current in streams, in the still shallow water at the edge of streams, in flooded stands of grasses and sedges, and on wet stream margins. Three features other than temperature seemed to be common to the places where the mites occurred: (1) the substrate was soft enough for them to burrow into and this was always provided by the algae and bacteria of the spring; (2) a zone of suitable temperature—equal to that selected by active adults—where some stable base for the lodgement of quiescent transforming stages was present; and (3) the presence of an adequate food supply. Tendipedid (Diptera) larvae were vigorously attacked by the mites and perhaps other nematocerous diptera larvae are taken as well. These larvae are among the commonest invaders of hot springs (Brues, 1928). These factors are almost universal characteristics of hot springs and did not limit mite occurrence in any of the situations studied.

Thus, *Thermacarus* appears to occupy almost any habitat if the daily temperature extremes lie between about 32° C and 48° C. Abundance, as evidenced by the ease of collecting, seemed related to the area available at the proper temperature. At locality 1 the extensive terraced flow is at the ambient temperature and the few defined stream flows enter the creek at temperatures above 50° C. Therefore, only limited narrow stream margins are available. Locality 4 originated with a small pool at 45° C and water leaving the pool cooled rapidly. Both areas supported small populations.

Large populations were found where there was either a large still pool of the proper temperature (Locality 2) or the major outflow proceeded far enough for the temperature to drop to well below 40° C (Localities 6 and 7). In the latter localities *Thermacarus* occurred in margins as well as out in the stream.

These data, based on less than five summer days' observations at each locality, suggest that *Thermacarus* responds to temperature and this was tested. Samples of mites were held in an ordinary half sphere kitchen sieve (6 inches in diameter) and this was placed in the current of the stream (with just the rim out of water so the mites could move freely but not escape). The net movements in the sample of mites stabilized very quickly with the proportions of mites upstream or downstream remaining constant. Once this equilibrium was established the counts usually varied less than 10%. It was then found that the proportion of individuals on the upstream half of the screen varied with temperature.

Samples reached an equilibrium in about 30 seconds and counts made after two minutes of exposure were an accurate measure of the upstream bias of the mite movements. If percentage of mites on the upstream half of the screen is well over 50% then there must be a dominant positive rheotaxis and if the percentage is greatly under 50% then the rheotaxis is negative. Males and females were tested separately but were found to be identical in their responses. The sequence of temperatures to which the mites were exposed did not alter the results.

Mites exposed to temperatures under 45° C show a strong upstream bias in their movements (fig. 11) but when temperatures excede 50° C the mites tend to move in the opposite direction. Somewhere between 45° and 50° C the mites shift from a negative to a positive rheotaxis and this simple behavioral response can account for the occurrence of *Thermacarus* within a limited temperature zone in hot springs.

Temperature records collected at the five localities listed above were from 33° to 50° C. At two of the localities (Localities 2 and 4) the mites were in a constant temperature pool. Where the mites could react to a wide range of temperatures, the mean temperatures at which mites were taken were 40.7°, 44.5°, and 40.2° C (Localities 1, 6, and 7 respectively). If current pushes mites down into a temperature zone slightly below the point where the rheotaxis reverses then the mean temperatures at which the mites were found supports the notion that the rheotaxis is the determining factor in the restricting of the mite to specific temperature zones in streams.

There is an undescribed species of *Thermacarus* that is structurally distinct from *T. nevadensis* but both species occur together and move similarly. The new species is of considerable interest because of its divergent anatomy but it is ecologically similar to the known species.

The Geography and Dispersal of Thermacarus

The species of the genus appear to require a minimum mean temperature of about 36° C and a range within 32° to 48° C. *T. thermobius* Sokolow is known from hot springs near Lake Baikal in Siberia (Sokolow, 1927) and the Tibet-Kashmir border (Vitzthum, 1940–1943). *T. nevadensis* Marshall (1928) is now known from 21 localities (Brues, 1928, 1932; and records in the Appendix) extending from north central Colorado and Yellowstone National Park westward to central northern California (roughly 760 miles east-west and 350 miles north-south). *Thermacarus* may have dispersed over this area while it was a facultative thermophile in water margins. If this is true, then the mite had virtually continuous avenues for dispersal under its own powers and under such circumstances host transport would be essential only if the time for dispersal was very short. Dispersal over this area is less likely to have occurred after the high temperature requirements of the active stages were developed.

The host is not known but larvae will attach to humans although the larvae do not engorge. If the host is a vertebrate

local dispersal may occur readily, but it is unlikely that the host could affect the dispersal of *Thermacarus* species over the entire range.

Ecology of the Genus Partnuniella

This genus of the family Protsiidae (fig. 14) contains four species of small soft-bodied vermiform mites. The two neotropical species, *P. walteri* Lundblad and *P. stalagmophila* Lundblad, were collected in streams but there is no data on their ecology (Lundblad, 1941).

The two species in hot springs of the western United States, *P. thermalis* Viets and *P. paucipora* Viets (the latter classified as subspecies by Viets, 1955–56) are shore zone residents and are found in the interstitial water of loose substrates but not on the surface. The mites were always found under stones or in the loose substrate on wet shores near the spring source. The temperature of the adjacent stream was almost always over 55° C but the temperature range at which mites of either species were found was from 30° to 47° C. Ninety-four specimens of *P. thermalis* from localities 6 and 7 occurred from 30° to 47° C with an average of 38.9° C. Twenty-five specimens of *P. paucipora* from locality 3 ranged from 33° to 45° C and averaged 38.2° C.

This range averages a degree or two lower than that of *Thermacarus* yet *Partnuniella* occurs nearer the spring source and this could be a function of size and locomotor ability. The mite is a very weak crawler and only the margin near the source provides a zone at the proper temperature range that is not likely to shift over a wide area. The zone is less than 5 mm wide but a tiny mite such as *Partnuniella* is not greatly restricted by this dimension. *Partnuniella* species are burrowing forms that may or may not restrict themselves to water-margins. It may be that high temperature requirements and limited locomotor ability force hot spring inhabitants to remain in the zones of stable temperature which, in hot springs, happen to be the margins.

The Origin of Thermophily in Water-Mites

1. The Genus *Tyrrellia*

Species of this genus are probably abundant throughout the United States for they have been found wherever proper collecting techniques have been used. Muddy or silty shores of small shallow ponds and of streams are the usual habitats but when one collects within a local region where the mite is abundant many apparently suitable habitats are unoccupied. All attempts to determine some peculiarity of the occupied habitats have failed so some set of unknown factors limit local occurrence. Whatever the precise set of conditions may be they are not limited to particular climatic zones, for species of *Tyrrellia* may be found throughout the United States.

Temperature data and mite behavior suggests that there is a continuous spectrum including both normal water-margin habitats and thermal water-margins. The tolerance of high temperatures, displayed by *Tyrrellia* is an adaptation necessary for occupation of water margins and not necessarily related to thermal waters. Further support for this idea comes from the fact that wherever there is a direct water flow from a hot spring *Tyrrellia* enter zones of lethal high temperature. The species of *Tyrrelia* are most favored in thermal habitats where the currents are not closely correlated with temperature zones such as the splash zones over terraces and no mortality was seen under these conditions.

These facultative thermophiles show that the locomotor adaptations to water margins require the mites to have temperature tolerances which are preadaptations to thermophily.

2. The Genus *Partnuniella*

The zoogeography of rheophilous water mites is poorly known because little collecting is done in a way that will capture these animals but what data there are suggest *Partnuniella* to be one of a group of stream associated mites that invaded North America from the Neotropics. Among the invaders were members of the genera *Partnuniella, Clathrosperchon, Tyrrellia,* and *Neoaxonopsis* and these represent a conspicuous Neotropical element in an area dominated by a Holarctic fauna.

Species of *Partnuniella,* like *Tyrrelia hibbardi* (Mitchell and Mitchell, 1958), appear to be derivatives from some invader that was widely distributed. Forms of the genus *Partnuniella* must have been distributed throughout the great basin area and it is perfectly reasonable to postulate that a climatic shift could affect suitable stream habitats in this area and leave relicts in hot springs.

This explanation must be looked upon as a tentative speculation for much data are needed on the ecology and behavior of the Neotropical species.

3. The Genus *Trichothyas*

The widely separated endemic populations of the genus may either be the relics of a once widely distributed form or else a polyphyletic assemblage produced by some common evolutionary trend.

In discussing temperature relations it was argued that there is ample indirect evidence that the species of the genus are all eurythermal animals. These data involve the location of all populations at low latitudes, the varied ecology of the species, the hot springs associations of two species, and the high temperature tolerance of *T. muscicola.* A known glacial relict, *Hydrovolzia gerhardi* Mitchell occurs in the same locality as *T. muscicola* Mitchell, 1953) and it is pertinent to note that *H. gerhardi* usually occurs on north facing slopes in waters about 18° C whereas *T. muscicola* is only on south facing slopes and exposed to a very wide range of temperatures.

If *Trichothyas* populations are relics there is no easy way to decide what the properties of the ancestor might have been for the populations seem to be equally local and restricted throughout the range of the genus. Obviously the ancestor did have to have the ability to spread over the northern continents and extend southward to Java, Central Africa, and Central America. If the mite could be successful over such an area it is difficult to imagine the conditions that might have brought about its extinction.

The only strong argument for the monophyletic origin of *Trichthyas* is the marked morphological similarity of the populations and the view that such a set of characters is not likely to have evolved independently. If this is accepted as a valid point then one is left with anomalies in geography and ecology that cannot be comforably explained.

Therefore it is important to reflect on the significance of the generic features of *Trichothyas.* Like other thyasid genera, *Trichothyas* is defined on the basis of the fusion or relative size of dorsal plates, differences in leg anatomy, and the proportions of the genital valves. *Tricothyas* (in Viets', 1955–1956, inclusive usage) contains a group of species possessing a particular kind of compound antero-dorsal shield, thick short legs bearing heavy setae, and thick narrow genital valves that leave the three acetabulae exposed.

The above-mentioned features of the body plates, legs, and chaetotaxy have already been shown to be well understood adaptations. The one feature that appears to be non-adaptative is the genital valve which may be a protective sclerite. The majority of the features of *Trichothyas* are demonstrable locomotor adaptations which makes their parallel evolution much more plausible. Since the adaptations of *Trichothyas* involve several different structures it is profitable to consider a mechanism that might produce

the various adaptations. The complete lack of developmental information makes any proposals highly speculative but speculations along these lines will be useful if they show the kinds of information that may explain *Trichothyas* evolution.

When the features shared by the species of the genus are analyzed for common elements it is apparent that each is the consequence of very heavy deposits of exocuticle and low length/width ratios. These things could result from slight changes in the timing and rate of exocuticle production. Presumably the cuticular tanning agents would be able to harden any additional exocuticle produced. Therefore it appears that an increase in exocuticle production might produce heavy short setae, thick coxae with broader apodemes for muscle attachment, heavy dorsal plates, and thick heavy genital valves. Subsequent induction of muscle development could produce large heavy coxal muscles.

Such changes in developmental rates could easily be selected for and if *Trichothyas* species did evolve from a *Thyas*-like ancestor on several separate occasions, there must be some conditions that tend to subject members of some ancestral population to selection favoring the evolution of water-margin adaptations.

Extant species of *Thyas* are walking or crawling forms presumed to be very similar to the ancestor of *Trychothyas* and most of the new world *Thyas* species are very occasionally seen to crawl in water margins. *Thyas rivalis* Köenike, found in the moss of cold springs, is slightly modified in the direction of crawling forms (Schwöerbel, 1959). If a population of thyasids resembling modern *Thyas* species did occupy both ponds and connecting streams some degree of genetic isolation could occur. Spatial separation would be of much less importance than ecology. The population, which must keep to the edges of the stream in order to avoid currents, would encounter very high mid-day temperatures in this situation. This high temperature may even differentially affect some developmental processes.

If mites in the two habitats are out of phase it may severely limit genetic exchange. Mating of the Thyasinae is probably similar to that of *Hydryphantes* (Mitchell, 1958) and, if so, it involves the deposition of short-lived spermatophores that are picked up by the females. Mature males deposit spermatophores regardless of the presence of females (Lipovsky *et al.*, 1957, Mitchell, 1958), thus, a developmental change correlated with habitat would have a greater than usual isolating effect if it affected the time of spermatophore transfer.

The final line of evidence has to do with the lability of the exoskeleton. Individuals of *Trichothyas* species show considerable variation and even asymmetrical fusion of plates occurs (Lundblad, 1942). All thyasid genera contain species that exhibit variation in the dorsal plates and the usefulness of dorsal plate geometry in species differentiation indicates that the plates have been subject to considerable evolutionary change.

Having discussed the probability of (1) most features of the genus *Trichothyas* being adaptive, (2) slight changes in time and rate of prospective exocuticle secretion being able to produce many of the generic features, (3) population segments of thyasids occurring along stream margins tending to be isolated from adjacent still water populations, and (4) the individual and specific variation in dorsal sclerites indicating a labile system, it is appropriate to see how these properties might help explain the origin of *Trichothyas*.

Any species of the genus *Thyas* can illustrate the morphology of a *Trichothyas* ancestor. Consider that this animal possesses the features common to the subfamily Thyasinae; it walks along the

bottom, has a variable set of dorsal shields, and probably transfers sperm to the female with no pairing. This animal may occupy a variety of habitats including streams where it must keep to the very shallow current-free margins. It follows that such an animal is often likely to have segments of populations partially separated from each other genetically in each generation, and, because these isolates are in particular sorts of habitats, subject to different selective pressures.

It has been demonstrated that freely interbreeding sub-populations of *Drosophila* may diverge along two different lines (Thoday and Boam, 1959). Because the mating system employed in this experiment insured a "forced gene flow" it clearly established that selection alone can bring about divergence. Another experiment with *Drosophila* (Knight *et al.*, 1956) showed that selection for intra-line mating of two genetically marked stocks produced a degree of genetic isolation in at least one of the stocks even though the virgin flies had a free choice of mates. Both these experiments produced positive results in a matter of twenty to thirty generations.

Because the dorsal plates of the thyasids are so variable it is tempting to think that the exocuticle production may be influenced by some environmental factor. If it were increased in stream habitats then the stage is set for the selection of phenocopies. Waddington's data (1953, 1956) shows that rigorous selection of an adaptive phenotype may be accompanied by the fixation of a genetic background for the character and this may have occurred.

Any attempt to specify the evolution of *Trichothyas* species in any greater detail would be a pointless speculation. It is quite enough to point out that thyasids in ponds and streams require very different sorts of adaptations and that mites of a single habitat are likely to breed with each other. The experiments cited above suggest that an animal in such a situation could diverge into genetically isolated species without the intervention of any physical barrier. Finally the plausibility of this as an explanation for the parallel evolution of *Trichothyas* is strengthened by the fact that selection acting on a single developmental process (exocuticle production) could produce the characters used to discriminate the genus.

4. The Genus *Thermacarus*

Arguments as to the history and origin of *Thermacarus* species very nearly parallel those used to explain the evolution of *Trichothyas* species. The ancestral *Thermacarus* is assumed to be a water margin inhabitant if these explanations apply but, of course, one must keep in mind that the adaptations of *Thermacarus* do not preclude its occupation of other habitats.

If the ancestor to *Thermacarus* species did inhabit water margins as *Tyrrellia* and *Trichothyas* species do today one can plausibly explain its ecology and occurrence. Species of the latter genera show that thermophily can be no more than the restriction of an extremely eurythermal animal to the upper part of its temperature range. Such eurythermy is directly associated with an easily recognized set of locomotor adaptations.

Therefore it is reasonable to postulate that the obligate water margin inhabitants but facultative thermophiles of the genera *Tyrrellia* and *Trichothyas* and the facultative water margin inhabitants but obligate thermophiles of the genus *Thermacarus* both occupy thermal waters as a consequence of having acquired high temperature tolerances as water margin inhabitants.

The most important property of such a *Thermacarus* ancestor is that it can disperse over a broad area and is virtually certain to include hot springs among the habitats occupied, thus eliminating the need for special means of dispersal. But there is difficulty in assigning to the an-

cestor both the ability to occupy a wide range of climatic zones and yet be labile enough to be destroyed in all but hot springs habitats. The alternative is to consider the possibility of parallel evolution. The arguments for these alternatives follow those given above for *Trichothyas*.

When the host of *Thermacarus* is known these views will have to be modified and the whole matter of dispersal may be explained but explanations for the evolution of thermophily will not be altered by data on host dispersal.

Summary

Species assigned to two unrelated genera, *Tyrrellia* and *Trichothyas*, have evolved a high degree of temperature tolerance as an adaptation for living in water margins and this physiological adaptation is closely correlated with adaptations in the locomotor organs.

Thermophily in these genera is facultative and represents an occurrence of individuals at the extreme end of the range of temperatures normally experienced in the margins of non-thermal waters.

Species of the genus *Tyrrellia* are so adaptable as to be able to distribute over a great range of climatic zones. Present day *Trichothyas* species occur as isolated relics, and it is conceivable that relic populations of *Trichothyas* could have come from a widespread ancestor but an ancestor connecting all the known isolates of *Trichothyas* would have to have been immensely successful and adaptable. This adaptability makes it difficult to imagine circumstances that would bring about the extinction of the ancestor.

Alternatively the characters of *Trichothyas* species involve shortened and heavy sclerites that are purely adaptive or related to processes that might produce the adaptive features. It follows that the complex of characters distinguishing *Trichothyas* species could have evolved independently in different areas.

The responses of thyasids to environmental conditions and the action of selection on individuals in different habitats could produce divergence within a thyasid population in the absence of external isolating factors. This can provide a very plausible model for the parallel evolution of *Trichothyas* species.

Species of the genus *Thermacarus* have the structure of water margin forms and all modern species are obligate thermophiles. There is good evidence that the mite could have evolved from a water margin inhabiting ancestor that was preadapted to thermophily. In accounting for the distribution of *Thermacarus* species problems identical to those considered for *Trichothyas* arise and one must admit two possible explanations: dispersal and subsequent extinction of some monophyletic ancestral group, or the polyphyletic acquisition of water margin adaptations.

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My long discussions with Kenneth W. Cooper were so productive that I wish to express particular appreciation for his lively and imaginative criticisms that were especially helpful in the development of evolutionary explanations. I am also greatly indebted to collegues at the George Reserve (University of Michigan), the University of Florida, and the University of California for much assistance and valued advice.

Appendix

1. "Hot Springs" 8 miles northwest of Steamboat Springs, Colorado, in the Routt National Forest. There are several outflows along the valley of Hot Springs Creek, those on the north side arise near the creek and flow right into the stream whereas those on the south side rise high on the slope. Some of the water is carried down the slope in distinct channels a few inches wide but most of the water splashes over the terrace-like surface. Outflow temperatures are about 60° C and none of the confined outflows reach a temperature below about 40° C before entering the creek. The splash down the terraces is cooled to air temperature.

August 1–2, 1958. Fauna: *Thermacarus nevadensis* Mars., *Tyrrellia cf. ovalis* Mars.

2. Hot springs by "Cleveland" 25 mi. north Preston, Idaho, on state route 34. The "town" of Cleveland is an abandoned store and right behind the store are four deep circular pools of warm cloudy water. The long activity here seems to have built up a broad oval terrace. Each pool is 30 to 50 feet in diameter and has narrow marginal shelf and then abruptly drops down several meters. Very little flow is apparent in any of the pools although bubbling does keep the water circulating and at a perfectly uniform temperature. The temperatures of the pools were 36°, 37°, 37°, and 42° C. A very small stream carries off the flow and its initial temperature is 36° C. This was Brues (1932) locality No. 92. August 4, 1958. Fauna: *Thermacarus nevadensis* Mars. (Found only in two pools. Temperatures 37° and 42° C.)

3. "Sunbeam Hot Springs" consist of two major outflows (70° and 73° C) and several smaller outflows that lie along the north bank of the Salmon River a mile west of the Sunbeam, Idaho Post Office on U. S. Route 93. Much of the water is confined to streams that meander a bit over the steep slope and cools only slightly for it runs only a short distance before being collected and drained under the road in a narrow culvert. Seepage and minor lateral flows wet a large area of the slope but there is no broad shallow flow over terraces. August 8–12, 1958. Fauna: *Thermacarus nevadensis* Mars. (1 specimen), *Partnunielia paucipora* Viets.

4. Hot springs on Loon Creek approximately six miles downstream (northeast) of the Loon Creek Ranger Station which is 18 miles north of Sunbeam, Idaho. Numerous springs rise along the east bank of the creek and the five upstream springs pour 55° to 64° C water directly into the creek with minimal marginal or splash zones of lower temperature. A short distance downstream there is a broad wet meadow in which there are several small springs that join to form a shallow stream. The upper "spring," a pool about one and one half feet in diameter, may be the outflow from a talus slope and it was at 45° C. The other springs were as low as 36° C and the temperature (mid-day) of the main flowing stream was 38° to 37° C. August 10–11, 1958. Fauna: *Thermacarus nevadensis* Mars., *T.* n.sp., *Partnuniella thermalis* Viets, *P. paucipora* Viets, and *Tyrrellia cf. ovalis* Mars.

5. Hot springs at Olene, Oregon. Just west of the bridge over the Lost River on the south river shore there are four or five small (a few inches in diameter) springs 50°–52° C in temperature. These were almost at the river level and flowed less than a meter over the bank before entering the river. August 22, 1958. Fauna: *Tyrrellia cf. circularis* Köen.

6. Hot springs 1.4 miles south of Lakeview, Oregon. Several outflows reaching a temperature of 83° C arose in a flat meadow and moistened a marshy area. Near the sources the streams meandered greatly and there was extensive adjacent wet shore. This area was drained by a well defined channel that later spread out to form a second marsh. The mid-day temperature of the stream just as it spread out in the marsh was about 38° C. August 24, 1958. Fauna: *Thermacarus nevadensis* Mars., *Partnuniella thermalis* Viets, *Tyrrellia cf. circularis* Köen.

7. Hot springs by Harney Lake approximately 25 miles south of Burns, Oregon. Some eight main flows of 55° to 65° C water arise just south of the lake on a gentle slope and contribute to a sheet of 55° C water that wets a broad surface for a short distance and then is collected by a sharply defined rapidly flowing stream. On a hot sunny day the temperature of water entering the stream was about 50° C and at the end of the stream (probably about 150 yards from the source) where the water floods over a marsh and immediately cools the temperature was 35° C. The wet margins throughout the areas of elevated temperatures were very narrow. August 26–30, 1958. Fauna: *Thermacarus nevadensis* Mars., *Partnuniella thermalis* Viets, *Tyrrellia cf. circularis* Köen.

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REPRODUCTIVE ISOLATION BETWEEN *DROSOPHILA TOLTECA* AND RELATED SPECIES [1]

STEWART E. ENSIGN [2]

The University of Nebraska

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Drosophila tolteca Patterson and Mainland 1944 is a member of the *D. affinis* subgroup (Subgroup b) of the *D. obscura* group of the subgenus Sophophora. It is distributed from Mexico to Bolivia (Miller, 1958b). Patterson and Mainland (1944) suggested, on the basis of morphological characters, that it is more closely related to *D. azteca* than to any of the other members of the *D. affinis* subgroup.

This study was undertaken to determine to what extent reproductive isolation exists between *D. tolteca* and other members of the *D. affinis* subgroup.

LABORATORY STRAINS

Single locality *Drosophila* strains used in this research were: *D. affinis,* Nebraska (Huskerville); *D. algonquin,* Nebraska (Humboldt); *D. athabasca,* Quebec (Laurentides Park); *D. athabasca,* Wyoming (Jackson Hole mix); *D. azteca,* California (Mather); *D. narragansett,* Nebraska (Halsey); and three strains of *D. tolteca,* Bolivia (Coroicó), Colombia (Villavicencio), and Nicaragua (Santa Maria de Ostuma). The strains designated "mix" were derived from two or more wild caught specimens, while all others were derived from single females isolated after capture.

In addition, certain strains were derived by crossing individuals from strains of different geographical origins. The Wyoming-Quebec (LP) strain of *D. athabasca* was established by crossing females from Jackson Hole, Wyoming, with males from Laurentides Park, Quebec. This strain was genetically marked by the sex-linked, recessive mutant gene "bright eyes" (Miller, 1958a). *D. athabasca,* Michigan-Quebec (SA), was started by mating females from the University of Michigan Biological Station by males from Sainte Anne de Bellevue, Quebec. The Colombia-Nicaragua strain of *D. tolteca* was derived by crossing females from a Villavicencio, Colombia, strain with males from a Santa Maria de Ostuma, Nicaragua, strain.

MATINGS

An attempt was made to determine the extent of sexual isolation existing between *D. tolteca* strains and strains of five other species by use of the "no-choice" mating technique. This involved combining males of one species with females of their own species, determining the insemination frequency, and comparing this figure with the insemination frequency obtained by the combining of males of this same species with females of a different species.

Newly-emerged flies were placed together and allowed to cohabit for ten or eleven days before the seminal receptacles of the females were dissected out and examined microscopically for the presence of sperm. The bottles in which the flies cohabited were kept in an incubator where the temperature was usually between 23° C and 28° C.

The results of this work are presented in tables 1 and 2. These tables show

[1] Contribution No. 322 of the Department of Zoology of the University of Nebraska.

[2] Aided by a Franklin E. and Orinda M. Johnson Fellowship from the University of Nebraska. Present address: Department of Microbiology, Yale University, New Haven, Connecticut.

TABLE 1. *Insemination frequencies of combinations involving* D. tolteca *and certain other members of the* D. affinis *subgroup. The following strains were used: Nebraska* affinis, *Nebraska* algonquin, *California* azteca, *Nebraska* narragansett, *and Nicaragua* tolteca

	Females inseminated	
	Fraction	Per cent
Intraspecific Combinations:		
D. affinis ♀ × *D. affinis* ♂	104/109	95.5
D. algonquin ♀ × *D. algonquin* ♂	110/125	88.0
D. athabasca ♀ × *D. athabasca* ♂	98/109	89.9
D. azteca ♀ × *D. azteca* ♂	101/105	96.0
D. narragansett ♀ × *D. narragansett* ♂	54/59	91.5
D. tolteca ♀ × *D. tolteca* ♂	104/109	95.5
Interspecific Combinations:		
D. tolteca and *D. affinis*		
tolteca ♀ × *affinis* ♂	25/105	23.8
affinis ♀ × *tolteca* ♂	31/107	29.0
D. tolteca and *D. algonquin*		
tolteca ♀ × *algonquin* ♂	12/104	11.5
algonquin ♀ × *tolteca* ♂	1/104	1.0
D. tolteca and *D. athabasca*		
tolteca ♀ × *athabasca* ♂	60/102	58.9
athabasca ♀ × *tolteca* ♂	19/118*	16.1
D. tolteca and *D. azteca*		
tolteca ♀ × *azteca* ♂	58/109	53.2
azteca ♀ × *tolteca* ♂	35/115**	30.4
D. tolteca and *D. narragansett*		
tolteca ♀ × *narragansett* ♂	13/98	13.3
narragansett ♀ × *tolteca* ♂	2/64	3.1

* Larvae produced.
** Adult hybrids obtained.

that, while sexual isolation did exist between the strains of *D. tolteca* and the other members of the *D. affinis* subgroup, the isolation was not absolute. *D. tolteca* mated with each of the other species, although with varying frequencies. Differences were also noted in the insemination frequencies of all reciprocal combinations of *D. tolteca* with each of the other species.

STUDIES OF HYBRIDS

A few larvae were observed in two of the 15 culture bottles containing combinations of Wyoming *D. athabasca* females and Nicaragua *D. tolteca* males.

As hybrids of *D. athabasca* and *D. tolteca* had not previously been reported, further attempts to obtain adult hybrids were made using mixed strains which were descendants of intraspecific matings of strains of different geographical origins. Females of one such mixed locality strain—Wyoming-Quebec (LP) *athabasca*—which was genetically marked by "bright eyes," a sex-linked recessive mutant gene, were combined with Colombia-Nicaragua *tolteca* males. From one mating bottle 27 female and 25 male hybrids were recovered. The hybrid nature of these individuals was evident as the females had normal eye coloration and the males, although they had bright red eyes, possessed morphological

TABLE 2. *Insemination frequencies of combinations involving* D. tolteca *and two strains of* D. athabasca, *mixed locality strains of both species being used*

	Females inseminated	
	Fraction	Per cent
Intraspecific Combinations:		
D. athabasca ♀ × *D. athabasca* ♂ Michigan-Quebec (SA)	57/62	91.9
D. athabasca ♀ × *D. athabasca* ♂ Wyoming-Quebec (LP)	47/54	87.0
D. tolteca ♀ × *D. tolteca* ♂ Colombia-Nicaragua	60/68	88.2
Interspecific Combinations:		
D. tolteca, Colombia-Nicaragua, and *D. athabasca*, Michigan-Quebec (SA)		
tolteca ♀ × *athabasca* ♂	17/60	28.3
athabasca ♀ × *tolteca* ♂	8/66	12.1
D. tolteca, Colombia-Nicaragua, and *D. athabasca*, Wyoming-Quebec (LP)		
tolteca ♀ × *athabasca* ♂	34/53	64.2
athabasca ♀ × *tolteca* ♂	21/59*	35.6

* Adult hybrids obtained.

characteristics intermediate between those of the parental species.

The hybrids (four to six in each combination) were tested in $F_1 \times F_1$ matings and in back crosses of both sexes with each of the parental strains, the experimental subjects being allowed to cohabit for a period of three weeks. In none of these combinations were any larvae produced, and no eggs were produced by the hybrid females. On the basis of these observations, both sexes of the hybrids were considered to be sterile.

The male hybrids appeared normal in size and general appearance except that the testis pigmentation was not so conspicuous through the ventral abdominal body wall as is usual in males of the *D. affinis* subgroup. At the end of the three-week cohabitation period, the reproductive tracts of the hybrids were examined and, although associated structures appeared to be normal, the testes were found to be extremely small compared with those of males of the parental species. The testes of some of the hybrid males were examined microscopically and no sperms or spermatocytes were observed in any of the specimens examined.

Since the first tarsal segments of the prothoracic legs of males bear sex combs which are important as a means of separating species in the *D. affinis* subgroup (Sturtevant and Dobzhansky, 1936; Patterson, 1943), these structures were studied in some detail in the hybrids. For purposes of comparison, prothoracic legs from Colombia-Nicaragua *tolteca* males and from Wyoming-Quebec (LP) *athabasca* males were also studied.

In 25 Colombia-Nicaragua *tolteca* males investigated by the author, the range of the sex comb tooth number was from six to eight, with an average of 6.7 ± 0.13 teeth per sex comb. This was a larger range than the six to seven reported for *D. tolteca* by Patterson and Mainland (1944).

D. athabasca may have as few as three and as many as six sex comb teeth (Miller, 1955), although the species was originally described as having four (Sturtevant and Dobzhansky, 1936). In 23 Wyoming-Quebec (LP) males of this

species, the author found a range of from three to five, with an average of 4.1 ± 0.11.

In the 22 *athabasca-tolteca* hybrid males examined, the number of sex comb teeth was five in nineteen of the specimens and four in the remaining three, with an average of 4.9 ± 0.08. Thus the hybrid average lies between the average numbers found in the *D. tolteca* and *D. athabasca* males examined.

A single sex comb tooth occurs on the second tarsal segment of the prothoracic leg in most of the *D. affinis* subgroup species (Patterson, 1943). It is almost always present in *D. athabasca*, but it is always lacking in *D. tolteca*. In the hybrids between the two species, the second tarsal segment tooth was present in 17 of the 22 males examined.

Sulerud (1958) suggested that the ratio of the first tarsal segment length over the second tarsal segment length (the f/s ratio) may be used to distinguish *D. athabasca* from *D. tolteca* males. In his study he found no overlapping of this ratio in the two species.

The average f/s ratio in the 22 *athabasca-tolteca* hybrid males which were examined in this study was 1.00 ± 0.013, with a range of from 0.85 to 1.13. Comparable numbers of males of the parental species were similarly investigated by the author. Twenty-three Wyoming-Quebec (LP) *athabasca* males had an f/s ratio of 1.24 ± 0.012, with a range of from 1.13 to 1.36. Twenty-five Colombia-Nicaragua *tolteca* males averaged 0.81 ± 0.008, ranging from 0.74 to 0.88. The average f/s ratio of the hybrids thus fell roughly midway between the values for the parental strains.

The hybrid females appeared normal in body size and proportions. The ovaries were dissected out and were found to be extremely small compared to ovaries of the parental species.

Observations of Mating Behavior

Mating behavior was observed in *D. tolteca* and the other *D. affinis* subgroup species. A preliminary study showed that the optimal conditions for observing copulation involved aging the flies for fourteen days in the incubator at 23° C to 28° C before making the observations.

The observations were made in chambers consisting of sections of glass tubing 20 mm high with an inner diameter of 12 mm. An agar plug 10 mm high filled the bottom of the chamber. The chamber was divided in half by a vertical piece of cardboard. A small quantity of food was smeared on the divider and a drop of yeast suspension placed on the food.

In order to prepare the flies for the observations, adults were separated according to sex within 24 hours after emerging and were aged in shell vials containing food slants.

After being aged for fourteen days, the flies were etherized and pairs to be observed were transferred to observation chambers, the male being placed on one side of the divider and one female on the other. Cotton plugs were put in place to restrict the flies to the chamber, and the chamber was placed on a glass slide.

Four to eight hours elapsed before the observations on mating began. This interval seemed desirable in order to give the flies time to recover from the effects of the ether. Sufficient humidity within the observation chamber was maintained by periodically adding a drop of water to the base of the chamber.

When the observations were to be made, the divider and the cotton were carefully removed, a $\frac{7}{8}$ inch square cover slip was placed over the top of the chamber, and the assembly was placed under the low magnification (13×) of a dissecting microscope.

To provide illumination, light from an incandescent source was passed through a round bottom flask filled with water. A sheet of onion skin paper between the flask and the observation chamber served to diffuse the light, bringing about conditions which seemed most favorable

to courtship and mating for the largest numbers of species. The light intensity at the observation chambers was about 18 foot candles as measured by a photometer.

Each pair was observed for thirty minutes. During this time the general pattern of courtship was studied and recorded and the duration of any copulation was timed with a stop watch. Ten to thirteen pairs were observed for each combination.

The mating behaviors of the six species were studied in conspecific crosses. From this it was possible to determine the general courtship pattern, although individual differences brought about exceptions to the behavior of the flies as given in this description. The major aspects of courtship under the conditions of this experiment and the number and duration of copulations observed in single locality strains are presented in table 3. The major features of *Drosophila* courtship have been described elsewhere (Miller, 1950b; Spieth, 1952) and will not be described in detail here.

Concurrently with the investigations of mating behavior in conspecific crosses, studies were carried out on the mating

TABLE 3. *Observations of mating behavior in conspecific combinations of six species of the* D. affinis *subgroup. The following strains were used: Nebraska* affinis, *Nebraska* algonquin, *Wyoming* athabasca, *California* azteca, *Nebraska* narragansett, *and Nicaragua* tolteca. *Figures under "tapping" indicate the proportions of the pairs in which tapping was observed*

Species	Behavior					
					Copulations	
	Angle of approach	Tapping	Wing movement	Circling	Number	Duration
affinis	45° from behind	6/11 55%	Both wings spread apart 90° and vibrated, also often vibrated one wing extended 60°	Circled only to rear from angle of approach	10	48″–2′35″ ($\bar{X}$ = 1′40″)
algonquin	Directly in front of female	10/12 83%	Extended both wings at an angle of 40° from body and vibrated them, also one wing extended 30°, then to 90°, vibrated, and back to 30°	Yes	7	3′5″–7′2″ ($\bar{X}$ = 6′3″)
athabasca	Directly in front of female	9/12 75%	Uniiateral; vibrated wing at 40° or at 90° or extended it 40° with no vibration	Yes	4	5′19″–7′5″ ($\bar{X}$ = 6′20″)
azteca	Directly in front of female	7/10 70%	Vibrated one wing extended at 45° angle, then while circling vibrated one wing held at 90° angle	Yes	8	4′30″–7′55″ ($\bar{X}$ = 5′52″)
narragansett	Directly in front of female	7/11 64%	Flicked one wing held 35° to 90° from body and elevated 20° to 30°, simultaneous attenuated movement of other wing	Yes	11	10′24″–19′39″ ($\bar{X}$ = 13′13″)
tolteca	45° from behind	11/12 92%	Extended one wing 90°, depressed posterior edge, sometimes vibrated the wing	Circled only to rear from angle of approach	10	2′45″–9′59″ ($\bar{X}$ = 6′4″)

TABLE 4. *Observations of mating behavior in interspecific combinations of* D. tolteca *females with males of five other species of the* D. affinis *subgroup. The following strains were used: Nebraska* affinis, *Nebraska* algonquin, *Wyoming* athabasca, *California* azteca, *Nebraska* narragansett, *and Nicaragua* tolteca. *Figures under "tapping" indicate the proportion of the pairs in which tapping was observed*

Species combination	Behavior						
						Copulations	
	Angle of approach	Tapping	Wing movement	Circling	Att. cops.	Number	Duration
tolteca ♀ × *affinis* ♂	45° from behind	6/10 60%	Both wings spread apart 90° and vibrated	No	0	0	
tolteca ♀ × *algonquin* ♂	Directly in front of female	7/10 70%	One wing extended 30°, then 90°, then back to 30°	Yes	52	0	
tolteca ♀ × *athabasca* ♂	Directly in front of female	10/10 100%	One wing extended 40°, usually vibration of wing	Yes	2	2	7′30″–8′27″ ($\bar{X}$ = 7′43″)
tolteca ♀ × *azteca* ♂	Directly in front of female	9/10 90%	Vibrated one wing held at 45° angle from body, then while circling vibrated one wing held at 90° from body	Yes	60	2	4′36″–5′22″ ($\bar{X}$ = 4′59″)
tolteca ♀ × *narragansett* ♂	Directly in front of female	10/11 91%	Bilateral flicking followed by unilateral flicking	Yes	73	1	10′25″

behavior in interspecific combinations of *D. tolteca* with five other species. These data are presented in tables 4 and 5. Females in these interspecific combinations displayed such mating deterrents as decamping, pushing against the males with their mesothoracic legs, fluttering their wings, extrusion of the vagina, and raising, twisting or lowering the abdomen.

DISCUSSION

The insemination frequencies observed between *D. tolteca* and other species of the *D. affinis* subgroup indicate that partial sexual isolation exists between *D. tolteca* and the other species studied. The fairly high frequencies of inseminations, however, were accompanied by few, or no hybrids. A situation of this nature in combinations of *D. affinis* females and *D. athabasca* males has been reported previously by Miller (1950a). It is noteworthy that *D. tolteca* exhibited some mating with each of the other species of the *D. affinis* subgroup, although other studies have shown little such interspecific mating between other members of the subgroup, e.g., Miller (1950a), Ensign and Miller (1957), Miller (1958a).

The hybrids obtained from *D. athabasca* females and *D. tolteca* males raise to seven the number of hybrids reported in the *D. affinis* subgroup.

The role of tapping in courtship behavior in the *D. affinis* subgroup species is an interesting one. Spieth (1952) in his studies of *D. affinis, D. algonquin, D. athabasca* and *D. azteca* stated that he had observed no instance in which courtship in these species was initiated without at least one tapping movement on the part of the male. Miller (1950b) reported that he found no tapping in *D. affinis* and *D. algonquin* although he looked closely for it. It should be men-

TABLE 5. *Observations of mating behavior in interspecific combinations of* D. tolteca *males with females of five other species of the* D. affinis *subgroup. The following strains were used: Nebraska* affinis, *Nebraska* algonquin, *Wyoming* athabasca, *California* azteca, *Nebraska* narragansett, *and Nicaragua* tolteca. *Figures under "tapping" indicate the proportion of the pairs in which tapping was observed*

	Behavior						
					Copulations		
Species combination	Angle of approach	Tapping	Wing movement	Circling	Att. cops.	Number	Duration
affinis ♀ × *tolteca* ♂	45° from behind	5/12 42%	Unilateral: extended one wing 90°, depressed posterior edge, sometimes vibrated wing; bilateral movement also	No	0	0	
algonquin ♀ × *tolteca* ♂	45° from behind	9/10 90%	Same unilateral movement as above; almost no bilateral movement	Circled only to rear from angle of approach	65	0	
athabasca ♀ × *tolteca* ♂	45° from behind	8/10 80%	Same unilateral movement as above; some bilateral movement	Rarely	0	0	
azteca ♀ × *tolteca* ♂	45° from behind	9/10 90%	Same unilateral movement as above; almost no bilateral movement	Circled only to rear from angle of approach	54	0	
narragansett ♀ × *tolteca* ♂	45° from behind	9/11 82%	Same unilateral movement as above; almost no bilateral movement	Yes	59	0	

tioned, however, that Miller did not use a microscope for most of his observations. In the strains observed during this investigation, tapping was observed in all of the combinations studied but *not in all of the courtships.*

This study was limited in scope since it dealt with only a few strains of the species involved, and some of these strains were "unnatural" in that they were derived by hybridization of geographically different strains. Nevertheless, interspecific inseminations were observed in all possible species combinations with *D. tolteca* and, moreover, some species hybrids were obtained. These findings indicate that the species are really very similar and closely related, despite their evident differences of morphological characteristics and geographical distributions.

SUMMARY

A study was made of reproductive isolation between *Drosophila tolteca* and a number of related species: *D. affinis, D. algonquin, D. athabasca, D. azteca,* and *D. narragansett.*

A series of "no-choice" mating experiments was conducted involving combinations of *D. tolteca* with each of the other species and intraspecific combinations of each of the six species. The insemination frequencies showed that sexual isolation is not complete in any combination, inseminations occurring in all combinations. However, some degree of isolation did exist between *D. tolteca* and each of the other species.

Hybrids were obtained from matings of *D. athabasca* females by *D. tolteca* males. Both sexes of the hybrids were

sterile, the gonads being poorly developed. The average ratio of the length of the first tarsal segment over the second tarsal segment of the prothoracic leg and the average sex comb tooth number in the hybrids were intermediate between those of the parental species.

Mating behavior was observed in *D. tolteca* and each of the other species. Courtship was initiated by tapping in some pairs in all the species studied, but not in every pair observed. Direct observations of mating behavior of *D. tolteca* with each of the five other species showed that courtship generally was not sustained up to the point of copulation.

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NOTES AND COMMENTS

EVOLUTION OF COMMUNICATION IN BEES AND ITS ROLE IN SPECIATION[1]

WARWICK E. KERR

Faculdade de Filosofia, Ciências e Letras de Rio Claro, Rio Claro, Est. S. Paulo, Brazil

Several families of bees have species that are either social or subsocial. Only the subfamily Apinae, however, possesses species that are known to have reached a social level sufficiently high as to have developed some method or methods of communication among their workers.

The Apinae social tribes, in phylogenetic order, are the following: Bombini (bumblebees), Meliponini (stingless bees), and Apini (honey bees). In these bees, the evolution of communication systems is indicated by the order of their increasing complexity, and by the parallel evolution of other characters of the species in which a given system of communication is found.

Ribbands (1953) suggested that exchange of food among adult bees was the first step toward communication. In fact, *Bombus* species neither engage in food exchange nor have a system of communication (Brian 1952, Free 1951). As far as nest structure is concerned, they are the most primitive Apinae (Kerr and Laidlaw, 1956).

A first evolutionary step may have occured when foraging was so intense that a field bee gave nectar to a house bee instead of delivering it directly to the storage pots. Such house bees, when they became foragers, may have been more attracted to flowers having the same odor as the nectar they received. Indeed, workers of the most primitive Meliponini known, namely *Trigona silvestrii*, alert their mates to look for an artificial source of food only when a drop of scent is added to the feeder (Lindauer and Kerr, 1958). The nest structure of these bees resembles very much the one found in *Bombus*. The communication of the location of either a source of food or a place to live by odor is a method found in all species of Meliponini and Apini.

A further step was reached when returned bees, while delivering nectar, ran excitedly in zig-zag movements knocking bees not engaged in field work with their heads or bodies. Such a method was discovered first by Lindauer (1956) in the Indian meliponid *Trigona iridipennis* and later by Lindauer and Kerr (1958) in ten species of Brazilian meliponids. By further work, I found this same mechanism to be present in eight other species, namely: *Trigona* (*Scaptotrigona*) *xanthotricha, Trigona* (*Scaptotrigona*) *postica II, Trigona* (*Scaptotrigona*) *postica III, Trigona* (*Trigona*) *hyalinata, Trigona* (*Trigonulla*) *muelleri, Trigona* (*Partamona*) *testacea,* and in the African species *Trigona* (*Hypotrigona*) *araujoi* and *Trigona* (*Meliponula*) *bocandei.*

One other method of alerting, either developed anew or brought from *Bombus*, is found in several (or all) species of meliponids. It is a special buzz that is perceived as vibration by the legs (Lindauer and Kerr, 1958). I found that the frequency of this sound varies with the species. Usually it lies between 464 and 484 vibrations per second for *Melipona quadrifasciata*, 326 and 348 for *T.* (*T.*) *jaty*, and is approximately 391 for *T.* (*S.*) *postica I.* These are only the frequencies of the most common sounds, the total range being greater than these presented. For instance, *Melipona quadrifasciata* sounds vary from 348 to 588 v.p.s. It may be that the ability to use sounds for communication has been lost in *Apis mellifera*, although it is very interesting to know that Hansson (1945) and later Frings and Little (1956) were able to stop activity of bees by subjecting them to simple sounds. Frings and Little (o.c.) caused bees to cease movement by subjecting the colonies to sounds between 300 and 1000 v.p.s. (Sounds between 300 and 800 v.p.s. were most effective.) *Apis mellifera* workers, therefore, have maintained the nerves (Shön, 1911) for perceiving vibrations similar to the ones perceived by stingless bees, but seem to have lost the ability to use them for communication. These sounds may not provide information concerning distance or direction, but are excellent for alerting other individuals.

It seems likely that the primitive Apini had three simple methods of communication: odor of the crop, zig-zag runs, and alerting buzzes. As far as we can see, further evolution of communication in the Apis species proceeded mostly from the zig-zag runs to the specialized dances. But even in these communicatory dances, three evolutionary levels were disclosed by Lindauer (1956). He found that *Apis florea* (which is morphologically the most primitive *Apis*) dances only in the horizontal

[1] Research reported in this paper was done with the help of the Conselho Nacional de Pesquisas (Brazilian Research Council) and the Rockefeller Foundation.

plane pointing straight toward the food source. *Apis dorsata* workers perform the dance on the vertical comb, but need to see the sun during the dance. *Apis mellifera* performs the so-called circle, sickle, and wagging dances (von Frisch, 1950), that are of amazing precision, inside the dark hive.

The species within the Meliponini have developed along two different paths. The *Melipona* species are the ones with the stronger buzz. In addition, *Melipona* species and some *Trigona* (for instance, *Trigona testacea*) release in the air great quantities of odor from their mandibular gland which attract other bees to a food source. The *Trigona* species are strikingly variable. Many of them remained primitive insofar as communication is concerned since they utilize only the three simple methods mentioned above. One group, however, has developed a particularly interesting method of communication. The scout bee, after having collected nectar or pollen for some time, acts to bring newcomers to this spot. To do this, as she flies from the flowers toward the hive, she stops frequently leaving on each stop an odor mark. The newcomers follow this odor path, which in *Trigona trinidadensis* can be as long as 900 meters, and reach the flowers in great numbers. Lindauer and Kerr (1958) found this method in the following species: *Trigona* (*Trigona*) *ruficrus*, *Trigona* (*Scaptotrigona*) *postica*, *Trigona* (*Geotrigona*) *mombuca*, *Trigona* (*Cephalotrigona*) *capitata*. Recently, in our laboratory, the following species were found to have the same communication method: *Trigona* (*Trigona*) *trinidadensis*, *Trigona* (*Trigona*) *hyalinata*, *Trigona* (*Scaptotrigona*) *postica II*, *Trigona* (*Scaptotrigona*) *postica III*, *Trigona* (*Scaptotrigona*) *xanthotrica*. The odor producing substance for the marks comes from a pair of mandibular glands (Lindauer and Kerr, 1958). This method is very precise and works better than the dances of *Apis*. Colonies of species having this mechanism occur in greater number than the others.

The average interval between two odor marks varies from species to species. In *Trigona ruficrus*, it is about 8 meters; in *T. capitata*, around 5 meters; in *T. trinidadensis*, 20 meters; and in *T. postica*, it is approximately 1 to 2 meters.

For the species of the subgenus *Scaptotrigona*, the odor mark system of communication, besides improving fitness of the species, has had a further evolutionary effect. Since the distance between two odor marks is around 1 or 2 meters, any river about ten meters wide would be big enough to constitute a geographic barrier for one of these species facilitating its break up into several new ones. Variability in the species of this subgenus is very great indeed. Two subspecies of *T. postica I* have been ascertained to belong to two different reproductively isolated sibling species, not crossing with each other nor being attracted to the odor marks of the others (they are called *T.* (*S.*) *postica II* and *III* for the time being). In addition *T. postica I* is extremely variable. It is very difficult to decide which of its varieties are species and which are subspecies, at least without a test of interbreeding. Recently Kerr and Stort (unp.) found that populations occurring North of the river Piracicaba are indistinguishable from ones located 40 kilometers apart; however, they are different from ones located in the South side, about 100 meters apart.

I wish to thank Dr. Walter Rothenbuhler very much for the trouble he had to put this paper in readable English.

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DIAGNOSIS OF THE CLASSES REPTILIA AND MAMMALIA

GEORGE GAYLORD SIMPSON

Museum of Comparative Zoology, Harvard University, Cambridge

The editor has asked me to comment on two papers (Reed, 1960; Van Valen, 1960) that are published in this issue. Both those authors reach, by different paths, the conclusion that the now almost universally accepted contents of the class Mammalia should be changed to include certain extinct animals hitherto classified as reptiles (nearly but not quite the same reptiles in the two proposals). Along with a number of others who have been informed of, or who have themselves thought of, this possible change, I am opposed to it, and I have agreed to appear here as a representative of those who support the current arrangement.

There is little disagreement as to the facts or as to the essential evolutionary interpretations of them. It has been known for generations that the mammal-like reptiles or the Therapsida (whose name includes the root for "mammal") are indeed mammal-like. Recent discoveries have added interesting details as to just what characters are mammal-like and as to approximately when they appeared, but the broad picture has not been changed. It has been believed by some for a shorter time, but still for more than thirty years now, that the line between reptiles and mammals, as usually drawn, was crossed independently by more than one lineage of mammal-like reptiles whose descendants thus became, by arbitrary diagnosis, mammals. Discoveries of the last few years have strongly confirmed that conclusion, as pointed out recently in two papers (Olson, 1959; Simpson, 1959), which apparently stimulated the present exchange of views.

The facts of progressive advance toward typical mammalian status and their interpretation in terms of evolutionary principles and of phylogeny are certainly of very great interest —far greater interest than the classification of the animals involved—but the present issue concerns classification, alone. Although based on scientific data, inferences, and principles, classification by its very nature involves a large, at times even a predominant, element of human judgment and ingenuity, in other words of art. The art of classification has certain canons that are so widely accepted as to restrict the play of fantasy, at least. I shall not attempt to discuss the canons in detail here (I am doing so in a book to be published by Columbia University Press), but only mention three kinds of criteria:

1. A classification should be consistent with a scientifically based set of principles and body of inference from evidence. Only in this sense, by consistency or inconsistency with its author's principles and inferences, can a classification be called "right" or "wrong." In my opinion, Reed's proposal is probably wrong by his own principles; Van Valen's is also questionable in this respect, but it is less inconsistent.

2. Even though there are always some doubtful cases, especially in paleontology, a classification should provide taxa in which a majority, at least, of the pertinent forms can be unequivocally placed on available data. In other words, a classification really should classify. Reed's and Van Valen's proposed classifications can be made to do this only by introducing considerations of doubtful or no relevance to their reasons for proposing new classifications.

3. Classification should be as stable as is consistent with the progress of the science and art. A widely accepted classification should therefore be changed only for compelling reasons, especially if it is clearly inconsistent with known facts or strongly supported inferences or if it has become definitely less meaningful or practical than a well-documented alternative. Reed's and Van Valen's proposals are to change classification admittedly almost universally accepted. Of course they think their reasons compelling; I do not.

Both Reed and Van Valen favor evolutionary classification, in some sense of the words; they base their arguments largely on the same, agreed inferences as to evolutionary events; and they reach almost the same conclusion. Nevertheless their stated taxonomic principles are so different as to be almost diametrically opposed. Reed believes (a) that classification should be strictly monophyletic, and (b) that the "fundamental basis for establishing a major taxonomic category" is "the establishment of some particular adaptive trend." He further thinks that the requirement of monophyly means that classification should be vertical, not horizontal, and he emphatically considers the recognition of grades in regular classification as inconsistent with this principle. Van Valen explicitly considers grade criteria as paramount and his proposal is to make the class Mammalia correspond with his conception of a mammalian grade, although in placing the exact boundary he brings in a criterion of convenience as well. He mentions possible attainment of monophyly only incidentally, and evidently considers it no drawback that the

Mammalia as he defines them may not be monophyletic.

Reed makes monophyly his prime criterion, but does not define it. It cannot be defined as derivation from one individual or mating pair, not because there is anything illogical *a priori* in such a definition but because it simply is inapplicable to most of the things actually being classified. Derivation from one species is also impractical, because (among other even more important reasons) a single species may itself be derived from two, a very frequent phenomenon in plants and not so rare among animals as some seem to think. At the other extreme, Beckner (1950), using other and more technically precise terms, defines monophyly as derivation of a taxon from another of the same rank. That is still too stringent a criterion to apply to species, and most taxonomists would consider it not stringent enough for such high taxa as orders or classes. The point is, however, that a criterion of monophyly that can actually be used in classification practically has to be relative to the rank of the taxon in question. In the paper (Simpson, 1959) to which Reed is reacting I suggested such a criterion: a taxon may be considered monophyletic if derived from one of next lower rank, or still lower. I was speaking of classes and would apply a *less* stringent criterion below about the rank of family. Some may prefer either more or less restrictive definitions of monophyly, but I think almost anyone experienced in classifying organisms at numerous different levels will agree that a fully practical criterion must be relative to categorical rank.

By that criterion, the class Mammalia as *now* defined *is* monophyletic. I regret that I obscured that point by using the term "polyphyletic" in a loose and even contradictory way in the paper in question (Simpson, 1959). That followed the vague current usage but undoubtedly caused misunderstanding.

Reed's way of meeting his criterion is to extend the class Mammalia down to and including "one"—unspecified and unspecifiable—"of the sphenacodont pelycosaurs." The authority cited for this source of the Therapsida is Olson (1959), but already Olson (*in verbis*, as also cited by Van Valen) inclines to think that the order Therapsida did not arise from one sphenacodont or even several but also from other pelycosaurs, and there was general agreement among specialists on that subject at the November, 1959, meeting of the Society of Vertebrate Paleontology. That outcome shows, at the very least, that an attempt to define Mammalia on this basis is extremely premature. Attempts to make the class Mammalia monophyletic by the strictest sort of nonrelative criterion could well end up by including in the Mammalia what are now, with good reason, called amphibians or perhaps fishes. I agree with Van Valen that even the inclusion of any pelycosaurs would be absurd.

Reed's principle that higher categories should begin with the establishment of some particular adaptive trend is also open to question. A few do, and probably more do not. Much of Reed's own work has been on a family (Talpidae) that did happen to start by, or rather with, such a trend. The order Artiodactyla started with a single key adaptation, quickly acquired, and with no further trend (Schaeffer, 1947). The order Primates had trends after it arose, but none clearly definitive of its origin (Le Gros Clark, 1959). What eventually became an amphibian adaptive trend clearly started in some of the earliest known true fishes or even before.

Reed considers the one definitive adaptive trend of the Mammalia to be toward endothermy. There is, however, no evidence at all that endothermy started in what he identifies as the monophyletic origin of the Mammalia, and some evidence that it did not. Even now and within the one group that everyone without possible exception considers mammalian, the Theria, endothermy is not completely established. Reed lists nineteen characters in addition to endothermy as common to Prototheria and Theria, hence in his opinion as evidence for including the Therapsida in the Mammalia and as adaptations dependent on or secondary to endothermy. Of these, no less than fifteen have no established relationship to endothermy, and at least five are known to occur also in animals that are *not* endothermic. (Some of them even occur in amphibians.) Even if, against all probability, there was an endothermic pelycosaur, it is most unlikely that we will ever know it or can ever either validate or apply Reed's definition. (Just to keep the record clear, I think it very likely that some late therapsids were at least imperfectly endothermic.)

Reed (1960) has quoted some of my remarks on the necessity for compromise between vertical and horizontal classification and I have elsewhere gone into the matter at greater length (Simpson, 1945, and *Principles of Animal Taxonomy*, in press). The fact that some compromise is necessary is really beyond argument, and opinions as to the way to make the compromise are sufficiently covered in dealing with other points, so I will not belabor the matter here.

Van Valen's grade principle is incomplete in practice, because in workable evolutionary classification it must be accompanied by some criterion of monophyly. By selection of characters, a grade taxon of birds plus mammals or even of cephalopods plus fishes could be erected, but those groups would be too poly-

phyletic for anyone. Pertinent grades usually are monophyletic by the criteria I prefer; practically all of Huxley's (1958) are. If the therapsids proved to come from more than one suborder of pelycosaurs, the question would arise as regards Van Valen's proposal, but as the matter stands now it is not crucial. As a matter of general principle, nevertheless, I cannot (and I think few will) subscribe to his principle of erecting higher categories *primarily* by adaptive status. (Although I think Mayr, Linsley, and Usinger, 1953, go too far when they exclude adaptive facies from consideration for most higher categories.)

The more important difficulty here, as I see it, is in the practical application of the grade criterion to this particular case. Van Valen puts the characteristics of the mammalian grade under three main rubrics—reproduction, intelligence, and activity—and discusses about a dozen (not precisely enumerable as distinct) more specific adaptations involved in those three. As Van Valen makes clear, at least by implication, those adaptations did not arise suddenly or simultaneously or at uniform rates. Very few are actually known in therapsids. There are practically no direct data except for very inadequate observations on gross brain structure, which was not mammalian, and fairly adequate observations on locomotor apparatus, which is nearly mammalian in some later therapsids. There is, I agree, indirect and somewhat equivocal evidence that some others of these adaptive characters were well along toward the mammalian grade among later therapsids. This is, however, a continuum and no one, including Van Valen, argues that any therapsid was on the most widespread or fully developed mammalian level in all these respects. The grade did not arise in one determinable step, as by special creation or by one of Goldschmidt's systemic mutations, or even by one adaptive complex, as, for example, did the order Rodentia.

Where, then, to draw the line? Van Valen's argument, unduly abbreviated (but it is published in full in this issue), is that later therapsids are nearer the mammalian *Typus* than the reptilian *Typus*. (The typological approach is, in my opinion, a real weakness here, but I shall not take space to discuss that point.) But when any group arises gradually, as mammals did, their immediate ancestors will always and necessarily share much of their *Typus*. The early therapsid *Typus* (if one must call it that) was certainly more like the pelycosaur than like the mammalian *Typus*, and one has only to approach from the other end to put all Therapsida in the Pelycosauria as perfectly good reptiles, which in *that* way they are. At a loss for a handy cut-off point within the well-documented Therapsida, Van Valen then feels constrained to call *all* therapsids "mammals," while admitting that more reptile-like forms are thus included in his "Mammalia." His "most reasonable stopping place" is largely provided by a break in record between early and late Permian, but even now that is filling in. There is, moreover, an equally marked break in record where we now place the reptile-mammal line. Using such breaks is, we hope, a temporary expedient, but in this case I see no obvious reason for abandoning one expedient for another.

In such a continuity, from undoubted reptiles (pelycosaurs for Van Valen, as for me) to undoubted mammals (Theria for both) one can draw a grade line about as well in one place as in another just by definitions of the grade. The Jurassic mammals by present definition were not as fully mammalian as the Cretaceous-Recent Theria, but were more nearly so than Triassic therapsids. Why not leave the grade line there, above the Triassic therapsids?

As for the ability of the classification to classify, Reed provides no better means than to begin the class Mammalia with the first animals that had a trend toward endothermy. The definition cannot be applied to any real animals in groups where the line must be drawn. Reed says, in different words, that he is not concerned with actually classifying the pertinent fossils but only in the principle of the thing. Everyone must be interested in the evolutionary and other principles involved (certainly I am), but a classification is not just a matter of principle, not even a means of *expressing* principles although it should be consistent with some principles. A classification has to be used to arrange real organisms, in short again, *to classify*, and Reed's proposal is not fitted to that purpose.

Van Valen sees this point, and he produces a practical definition of mammals as synapsid tetrapods with a small quadrate, no supratemporal, and a basic phalangeal formula of 2·3·3·3·3. That is a definition just as arbitrary as the one it is meant to replace and, if anything, less significant. It has no direct and only extremely doubtful indirect relationships to what Van Valen considers the real definition of mammals in principle: by reproduction, intelligence, and activity. The data of observation in his practical definition are not good or perhaps even suggestive evidence that his theoretical definition is met. As Van Valen notes, his "second and third criteria are not known in many instances; their use here is permitted by our ignorance." As a matter of fact, they are not known in *any* of the forms now called Mesozoic mammals (as distinct from therapsids and so-called ictido-

saurs). Van Valen also notes that the first is "difficult to define objectively." In most cases this would become our only practical criterion if Van Valen's definition of Mammalia were adopted. It is really impossible to say just when a quadrate becomes "small," but in most or all known cases it is possible to say when it *ceases functionally to be a quadrate* (i.e. no longer fully suspends the lower jaw). That is the point most closely definable by the quadrate, and that is where the line between reptiles and mammals has hitherto been drawn.

The final point, that of prevailing usage, is a touchy one to argue. It is so easy to appear to be (or to be accused of being) merely an old fogey defending the *status quo* against the cause of progress. Certainly changes should be made if they are in fact progress. It would be stupid to defend retention of the phyla Vermes and Radiata (Reed's examples) just because they are old. But it would be even more stupid to discard, say, the order Perissodactyla just because it is old, too. A great many different classifications could be based with full consistency on our present knowledge. It would be chaos if we did not agree to keep the ones in widest use *as long as other things are equal.* I have not argued and do not argue that classifications should not be changed, and like every other working taxonomist I change my own continually. I do insist that they should be changed only when there is good evidence that the change is for the better. For the reasons that I have now stated, not in full by far but probably sufficiently, I am convinced that the changes suggested by Reed and by Van Valen are not for the better.

I therefore propose that we continue to define mammals, for practical purposes of classification, by possession of a single bone in the lower jaw, articulating directly with the squamosal, and by presence of three auditory ossicles. In transitional groups this complex did not evolve all at once, and for purposes of drawing a *precise* line in them the criterion of a dentary-squamosal joint seems to me most practical. That criterion excludes all of the pelycosaurs and most, at least, of the animals now called therapsids.

Although I feel that inclusion of the Therapsida, and *a fortiori* of any pre-therapsids, would not improve on the present arrangement and therefore should not be adopted, there is still another alternative that seems to me clearly preferable to those suggested by Reed and by Van Valen. Whether that alternative is also distinctly preferable to the current classification is an open question, but it is at least one that should be seriously considered by those directly concerned with classifying the Mesozoic mammal-like vertebrates. The alternative is to make the Mammalia coextensive with the Theria of present classification, comprising the Jurassic Pantotheria and the Cretaceous to Recent Metatheria (marsupials) and Eutheria (placentals). There is little doubt that these groups are more closely related to each other than to any others contemporaneous with them at any one time.

The Theria are monophyletic in origin at an unknown but evidently reasonably low level, below the ordinal level and probably somewhere around the family level, possibly even lower. Thus if a rather low relative level of monophyly is sought, this is much the most practical way to attain it on the basis of animals now known. It is also a reasonable supposition, not open to demonstration at present, that the earliest Theria, i.e. the Pantotheria, were nearest to fully mammalian grade of all Jurassic animals. The practical definition cannot, however, be put in grade terms. It can be made completely precise phylogenetically on the basis of tooth and jaw structure: there is now no serious doubt as to what animals do and what do not belong to the Theria. The definition is also nonarbitrary as regards animals now known. Doubtless it will have to become arbitrary if or when early Jurassic or late Triassic therian ancestors are found or identified, but that is a problem for the future.

The main drawback of this proposal, apart from its also being contrary to current usage, is that it would leave out of the Mammalia a number of splinter orders that would then be very difficult to place satisfactorily: Triconodonta, Docodonta, Monotremata, Symmetrodonta, and Multituberculata. Late members of those orders had in many, even in most, respects reached the mammalian grade. Tritylodonta and Ictidosauria were not so far along in grade evolution, but were only a little behind. Reference of the former five orders, at least, to the Reptilia would thus be quite anomalous from this point of view. Their placing within the Reptilia would also be a problem, but they could be reduced to subordinal status in the Therapsida or retained as separate synapsid orders of therapsid origin. They might be left *incertae sedis* as to class, but that would be highly unsatisfactory when so many groups, some of them very well known (the monotremes, for one!), are involved. Because of these questions, I continue to recommend the *status quo,* but if a change does eventually seem desirable this is a more promising approach than the ones taken by Reed and Van Valen.

The class Mammalia did arise through trends, as Reed emphasizes, and these trends were to some extent already becoming established in the Therapsida. The trends included one toward endothermy, but in my opinion

they were multiple and the others were not all secondary or consequential to endothermy. It is also certainly true, as Van Valen emphasizes, that there is a mammalian adaptive grade distinct from a reptilian grade and that this is one of the reasons for recognizing mammals and reptiles as different classes. It is further true—it is almost a truism—that the reptiles in and nearest to the mammalian ancestry foreshadowed the mammalian grade to some extent. But the mammalian grade arose very gradually and is definable only in terms of numerous eventually associated characteristics that evolved at different times and with different rates. Those are agreed conclusions pertinent to the science of evolution. What is then needed is judgment as to the respective roles of that science and of the art of classification.

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THE ANTECEDENTS OF MAN [1]

EDWIN H. COLBERT

The American Museum of Natural History and Columbia University, New York

Sir Wilfred Le Gros Clark, who has devoted his attention during many years to the problems of human origins and human evolution as documented by many lines of evidence, speaks with the authority of a dedicated scholar whose vast experience and clear insight have contributed much to our understanding of a complex and difficult subject. Consequently the appearance of his latest book, based upon the Munro Lectures delivered at the University of Edinburgh, is an event of particular significance to students interested in the evolution of man. The numerous readers who pick up this new volume with eager anticipation will not be disappointed.

It is above all a balanced survey, for, as intimated above, Sir Wilfred interprets data from various lines of evidence; he is not one to ride a particular hobby or to be unduly influenced in any one direction, a weakness peculiar to so many students of primate evolution. For example he gives particular attention to the teeth in fossil and recent primates, because the teeth in these animals are very significant in establishing taxonomic and phylogenetic relationships, and because they are the most abundant of our fossil evidence, yet he does not allow any preoccupation with the dentition to outweigh other anatomical and paleontological evidence. This gives to the work a validity that will make it an outstanding reference for many years to come.

At the beginning of the book Sir Wilfred outlines the bases for evolutionary fact and theory, spelling out briefly certain facts and discussing derived principles in the fields of comparative anatomy, embryology, paleontology, terminology and geological time that are fundamental to a subject involving important data from both recent and fossil materials. Then, after discussing the classification of the primates he proceeds to follow through a sequence of forms, ranging from the most primitive primates to man, outlining quite clearly the evidence for evolution as based upon the dentition, the skull, the limbs, the brain, the special senses, the digestive system and the reproductive system. He concludes the book with a consideration of evolutionary radiation among the primates.

[1] Clark, W. E. Le Gros, 1960. The Antecedents of Man. vii + 374 pp., 152 figs. $6.00. Quadrangle Books, Chicago.

Clark emphasizes in this book a fact pointed out a century ago by T. H. Huxley, namely that the primates are rather unusual among mammals in that so complete a series, from primitive to advanced types, has been retained among the living representatives of the order. Thus even without the fossil evidence we are able to obtain a reasonably accurate picture of the progression of primate evolution, from the tree shrews that have shown little change since early Tertiary times to the modern monkeys, apes and man. Professor Clark finds the explanation for this in primate ecology; these mammals throughout their history have been primarily arboreal animals, and in the protected havens of the tree tops many primitive forms have persisted with little change along with their advanced descendants. This is at the same time a fortunate and an unfortunate evolutionary circumstance for the study of a group so important and interesting to us, because while it has preserved a nice anatomical series, it has militated against abundant fossilization, so that the paleontological record of the primates is correspondingly sparse. Professor Clark gives careful attention to the fossils, and supports the known fact that the evidence of paleontology can be correlated to an amazing degree with that of comparative anatomy.

He fully agrees that the tupaioids are true primates, as indicated by the fossil evidence and by a close study of living examples. This conclusion is further reinforced by the evidence of the parasites, for as Clark says "it is thus of peculiar interest that a genus of ectoparasites *Docophthirus,* which has been recorded from tree-shrews, is apparently quite closely related to the genus *Lemurphthirus* which is characteristic of the African lemurs." Here one sees an added advantage of having an evolutionary sequence through time approximated by living survivors on the various branches of the evolutionary tree.

Tarsius, the spectral tarsier of the Philippines and its various fossil relatives, have for many years been the subjects of taxonomic contention. It is Clark's considered opinion that the tarsioids show some very significant resemblances to the higher primates, so that if there are not direct lineal relationships between the two groups it may be reasonable to think that both were initially derived from closely related ancestors. The lemurs, by way of contrast, show through their long paleontological history broad evolutionary trends along certain directions, exemplified with but one exception by the end products—the existing lemurs. The exception is the aye-aye, *Daubentonia,* which occupies a rather isolated evolutionary position in relation to the other lemuroids.

The various monkeys were well established by Miocene times, and since then have followed evolutionary trends in which brachiation was a controlling factor. "It is possible with some reason [says Clark] to argue the thesis that the whole group of cercopithecoid monkeys represents a specialized sideline of evolutionary development which branched off from the main line of hominoid evolution towards the end of the Oligocene, and which followed an aberrant course in its dental specialization and in other features. . . ." And this is certainly a refreshing way of viewing the problem. Sir Wilfred, however, gives full recognition to the more usual interpretation, that "it would also be true to say that the anthropoid apes passed through a cercopithecoid phase of evolution." However that may be, Clark recognizes that small anthropoids had appeared in the Oligocene epoch, as exemplified by *Parapithecus* and that the beginnings of hominoid evolution might in a sense stem from such a beginning. Certainly during Miocene times there was a varied array of apes, to use a very general term from some of which the modern great apes were derived, and from others the first hominids. In this connection it should be said that Sir Wilfred is not greatly impressed by any evidence that purportedly might point to hominoid relationships for *Oreopithecus,* a genus that has been given much attention in recent years as a possible human ancestor.

The South African primate *Australopithecus,* of early Pleistocene age, is becoming known from increasing fossil materials, which show that it was a truly upright form with hominid teeth but with a very small brain. It represents a stage in evolution not far removed from the line leading to *Pithecanthropus,* this in turn being close to the ancestry of Neanderthal and modern man. In this sequence Sir Wilfred sees the early Neanderthals as being comprised of a rather variable population which eventually crystallized into two morphologic types. In one of these types, the characteristic Neanderthal man, there was a "secondary retrogression" that emphasized primitive characters in the skull and skeleton; the other type led to modern man.

Such are some of the important points brought out in this excellent and well illustrated summary of human evolution, a work that can be highly recommended, not only to those primarily interested in this subject, but also to evolutionists working in other fields.

FISH EVOLUTION IN LAKE NYASA

GEORGE SPRAGUE MYERS
Stanford University

Fryer's consideration of evolution in Lake Nyasa (1959) illuminates many points in the evolution of lake fishes and marks a great step forward in the study of endemic lake faunas. However, it also brings up some points which have not yet been clearly understood.

While I agree that the more easily perceived specializations of endemic species flocks in large lakes appear to be largely trophic in nature, some specializations almost certainly are not trophic. We are barely at the beginning of an understanding of the astounding diversity of fish adaptions and specializations, many of which are non-trophic and may be scarcely if at all indicated by external morphology or stomach-content analysis. Even observations of the living fish, unless made intensively for 24-hour periods throughout the year, may not uncover some important specializations. For example, we may cite much recent work on fish sounds and communication, species differences in the electric field with which mormyrids surround themselves, the mucous "sleeping capsules" of parrot fishes, the precision with which certain species select an exact type of substrate for breeding sites, the marvelous but differing ability of fishes to detect excessively dilute chemical traces, etc. Specializations of these or other non-trophic types may go completely unrecognized for a long time, even in relatively well studied fishes. In other words, we know very little about the real daily and seasonal life of most fishes, and to assume that the specializations of any considerable number of species of fishes are mostly trophic, or mostly anything else, is seldom fully warranted. In my Lanao paper (Myers, 1960) I refrained from speculation in regard to the kinds of specializations exhibited by endemic lake fishes, preferring to refer only to the uniqueness of known morphological characters.

For much the same reason, I must urge caution in the acceptance of Fryer's thesis that fish habitats in Nyasa are not diverse. When I read my Lanao paper (Myers, 1960) at the London Zoological Congress (including the aphorism that biologists are, unfortunately, not aquatic vertebrates), E. B. Worthington remarked that diving with an aqua-lung in Lake Tanganyika had given him a new conception of the diversity of Tanganyika fish habitats (see Myers, 1959: 152). To say that any subaquatic area forms a very uniform fish habitat on the basis of brief observation, even when aided by the common types of limnological sampling, is, I believe, not wholly justified *from the viewpoint of the fishes concerned*. All we know about the ecology of most lake species is what they often eat, where and how they are usually caught, and some gross information on the general type and time of breeding. Their ecology may involve a great deal more than this.

Fryer's observation that plankton feeding and the shoaling (schooling) habit go hand-in-hand in Nyasa cichlids is most interesting but it is so much to be expected that only the absence of this correlation would be remarkable. Except for minute fishes to which plankters are large prey, and for large plankton-feeders such as *Polyodon* and *Cetorhinus*, I know of no fish anywhere which strains out plankton with fine gillrakers that does not habitually school. Even *Polyodon* and *Cetorhinus* school at times for certain purposes, possibly not connected with feeding. The schools of basking-sharks off the Irish coast and elsewhere are well known.

Fryer's suggestion that the schooling behavior of some Nyasa cichlids may be a sort of behavioral neoteny is even more interesting, but cannot be proved until a good deal more work has been done. Investigation of schooling behavior has been carried out on relatively few species of fishes and there are apparently notable differences in such behavior as developed in different fish groups. The schooling of herring-like fishes and of various Cyprinidae, for example, is different in many ways. My colleague, Dr. Stanley Weitzman, has reminded me that there is seasonal and temperature-controlled schooling in centrarchid fishes, which are in many ways similar to cichlids, but this may not be true of Lake Nyasa cichlids. However, if the schooling of adults is "behavioral neoteny" among Nyasa cichlids, one or more important changes in the behavioral patterns of cichlids may be involved.

Certain basic patterns in the breeding behavior of cichlids are rather rigidly fixed, and appear to be less plastic than any morphological characters within the family. A large part of the elaborate courting, spawning, and parental-care ritual of many and presumably all Cichlidae is stereotyped to a remarkable degree (Baerends and Baerends-van Roon, 1950). I have twice had a female of the West African *Pelmatochromis kribensis* mate and spawn with a male of the Central American *Cichlasoma octofasciatum* in one of my own aquaria, indicating that even in a cross-mating of these distantly related and strikingly different forms,

the complex chain of behavioral releasers and responses inherent in cichlid breeding behavior could proceed to successful spawning. Moreover, the fertilized hybrid eggs were guarded until the cessation of embryonic development 36 to 48 hours later. In fact, the principal dichotomy of cichlid breeding behavior is between the nesting and the oral-brooding forms, the evolutionary relationship between which I have already pointed out (Myers, 1937).

Apparently all young cichlids school, whether they be oral brooders or not; I have personally observed this behavior in perhaps 30 species from America and Africa. However, it is now well known that the schooling of young cichlids, both nesters and oral brooders, is strongly oriented toward the body of one or both parent fish (Noble and Curtis, 1935; Baerends and Baerends-van Roon, 1950). At a certain point, after the young have been schooling with the parents for some time (and taking refuge in the mouth in oral brooders), both the relationship and the schooling definitely break up, and the young scatter. Perhaps this is due to the loss of high nuptial coloration in the parents. The scattering has happened with all the cichlids that I have studied, and it appears to occur generally, in both nesters and oral brooders. It seems possible that orientation toward the parents might become definitely prolonged in some species, but if so we would expect to find some schools composed of adults and smaller young. The finding of such heterogeneous shoals would definitely be indicative of behavioral neoteny, but if so, this would break a general rule of fish schooling or shoaling—that individual schools of fish are almost invariably composed of individuals of approximately the same size. I doubt that the Nyasa cichlid schools are of this heterogeneous type. On the other hand, if the schooling young lose parent-orientation *yet do not scatter*, an apparently rigidly developed behavioral pattern of cichlids would be broken. It would be interesting to know what really happens during the growth of these schooling, plankton-feeding, Nyasa forms. Until we do, Fryer's suggestion of behavioral neoteny must remain merely a suggestion, albeit a most interesting one.

Fryer's emphasis on the permanence of the lake environment, in contradistinction to the impermanence of the stream environment, as a factor in the extreme specialization of Nyasa cichlids, is a highly debatable point. That stream environments, and indeed, most lake environments, are usually transient, in comparison to the environments of older lakes, is true in one sense and not in another. Perhaps a better statement of ecological difference would be that lake habitats are seldom periodically disturbed (save in the shallows of fluctuating lakes), while those of streams usually are. Stream environments are often permanent, even in the geological sense, but are usually periodically disturbed, or even moved upstream or downstream, by floods and freshets. But the fishes, even very small species, survive these periodic disturbances, and, if displaced, return to the accustomed habitat. I have observed a small school of minnows (*Dionda episcopa*) in a small, disconnected, sandy pool in a Texas stream, seen the stream rise at that point to a swift torrent 12 feet deep and 200 feet wide after a desert rain, and returned two weeks later to see the same school in the same disconnected pool, including at least one identical individual seen before and recognizable by a fin-defect. Many North American fluviatile species are very narrowly adapted to sandbars, the gravel of riffles, or other distinctive biotopes, which, in country of low relief, may be exceedingly permanent features of the streams, in the geological sense. Moreover, the general fluviatile habitat of the larger tropical continental lowlands is usually accepted as one of the most permanent on earth.

That some of the narrow food and habitat specializations of Nyasa fishes is due in part to the lesser disturbance of lake habitats may be true, but Fryer's attempt to bolster this theory by a reference to the lack of environmental specialization amongst British freshwater fishes is most unfortunate. In fact, his statement that "freshwater fishes are, in general, relatively unspecialized" is simply not generally true except in what we might call atypical fish faunas. If he had compared the specializations of Nyasa cichlids with those of the fishes of the Zambesi River, from which much or all of the Nyasa fish fauna must have come, his comparison would have had some weight. That it is quite off the point is easily shown.

The fresh-water fish faunas of western Europe, Siberia, most of Canada, Alaska, Patagonia, and Chile are all depauperate, that of Britain especially so. Amongst the members of any such fish fauna, trophic and habitat specializations are almost invariably broad. In the far richer temperate fresh-water fish faunas (e.g., of north-central China, the rivers of Ohio or Virginia, or the vicinity of Buenos Aires) a river is usually inhabited by five or ten times as many species as those of a comparable British or Canadian stream, and many narrow habitat and trophic specializations are obvious. In tropical continental streams, the number of species is often or usually much larger than in any known lake fauna, and narrow trophic and habitat specializations are the rule. There are over 300 species of fishes in the Ganges Basin, probably over 200 in the Niger and 200 in the Nile (outside its headwater lakes), and perhaps over 500 in the Congo system (outside

Lake Tanganyika). In a minor South American river, the Essequibo in British Guiana, the fish-fauna probably totals between 250 and 300 species. Eigenmann got between 70 and 90 species in a single haul of a 100-foot seine in the Essequibo, and collected 60 species from a forest trickle too small to be called a brook (Eigenmann, 1912). After many years of study of the Amazonian fish-fauna, I estimate the number of fish species that must eventually be found to occur within a 20-mile radius of Manáus, in the lowlands of the middle Amazon, to be almost certainly in excess of 700. While I am unable to cite even approximate figures for the Zambesi and Shiré Rivers, from which Nyasa was populated, I do know that the Zambesi fish fauna is large, poorly explored, and poorly studied.

In fact, when the ecology of the fishes of the larger continental streams of tropical Africa, Asia, and America is carefully investigated, it seems certain that narrow specialization will be as clearly evident as it is amongst the endemic fishes of Nyasa and other large lakes. But the specializations will be found to be distributed through the species of many groups and not concentrated mostly in one dominant family, as they are in the larger endemic lake faunas (Myers, 1960).

One of the most promising future lines of attack on the problems of fish evolution in Nyasa would appear to be a careful investigation and comparison of the entire fish-fauna and its ecology with that of the Zambesi River, from which the lake was populated. The same may be said of Lake Tanganyika and the Upper Congo. Such a comparison ought to be exceedingly illuminating, and would provide a much better basis for theorizing than we now have.

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EVOLUTION OF FISHES IN LAKE NYASA

Geoffrey Fryer

East African Fisheries Research Organization, P.O. Box 343, Jinja, Uganda

While some of the comments of Professor Myers (1960) on my paper concerning evolution in Lake Nyasa (Fryer, 1959b) may lead to a clarification of certain phenomena, most should be countered.

Myers agrees that "the more easily perceived specializations of the endemic species flocks in large lakes appear to be largely trophic in nature" but suggests that "some specializations are almost certainly not trophic." As no one has denied this the comment would appear to be gratuitous, and in fact several non-trophic specializations of endemic Nyasan cichlids have already been studied, e.g. mimicry (Trewavas, 1947), nest building and other breeding habits (Lowe, 1952; Fryer, 1956, 1959a), burrowing in sand, feigning death (Fryer, 1956) and so on. All these, and others, are of importance in the lives of the fishes concerned and must have played a part in evolution. Nevertheless, when one can plainly see an array of strikingly adaptive and obviously important specializations such as mouth structure and dentition (oral and pharyngeal) which often profoundly modify cranial architecture and musculature, and which are intimately related to the collection and manipulation of food (a prime necessity of life) and to the general ecology of the species concerned, one must not ignore them and seek for cryptic specializations. To do so is bad ecological practice. In fact to follow Myers who believes that "to assume that the specializations of any considerable number of species of fishes are mostly trophic, or mostly anything else, is seldom fully warranted" would be to close one's eyes to the most obvious adaptations of the endemic cichlids, not only of Lake Nyasa but of all those African lakes in

which species flocks exist. Further, to refrain "from speculation in regard to the kinds of specializations exhibited by endemic lake fishes" and "to refer only to the uniqueness of known morphological characters" is to divorce structure from function and therefore render it meaningless from the point of view both of ecology and evolution. For example, to describe the mouth structure and dentition of the Nyasan *Petrotilapia tridentiger* Trewavas and the protractile mouth of *Lethrinops furcifer* Trewavas is of little value unless one relates these structures respectively to the scraping of algae from rocks, and the sifting of chironomid larvae from sand by a method which could scarcely have been deduced from structure.

Myers finds it difficult to accept the fact that fish habitats in Lake Nyasa are not diverse. Everyone will agree that "to say that any subaquatic area forms a very uniform fish habitat on the basis of brief observation, even when aided by the common types of limnological sampling is, I believe, not wholly justified from the viewpoint of the fishes concerned," but this implies that my remarks were based on brief observations. Although no aqualung was available to me, I did employ a glass-fronted mask which, because of the exceptional clarity of the waters of Lake Nyasa, enabled me to make many underwater observations over a period of some two years, as explained in my main paper on the subject (Fryer, 1959a). The observations were therefore not casual, and as I feel myself more qualified to judge than one who is unfamiliar with the lake, I must reiterate, as a statement of fact, that, although I cannot speak from the viewpoint of the fishes concerned, the rocky and sandy shores of Lake Nyasa are very uniform habitats, and that, as these two major types dominate the littoral zone, the number of habitats is few. Within each of these habitats of course exist various niches, some of which have been investigated in some detail. I emphasized the co-existence of many species per habitat which is a natural consequence of the paucity of habitats and can only repeat that in Lake Nyasa the situation is one where a few habitats are repeated many times and that many of the endemic cichlid fishes are minutely specialized for the exploitation of niches within these habitats. If there are many habitats why are the specializations of so many fishes directed to the same ends such as rock scraping and plankton eating?

Myers has missed my point about plankton feeding and shoaling. That these habits often (but not invariably) go hand in hand among fishes was, I thought, so well known as to require no emphasis. The important point is the concomitant occurrence of these habits in endemic fishes which indicates an as yet indefinable *something* which binds these two tendencies and will not usually permit the evolution of non-shoaling plankton-feeding species. What that *something* is remains to be discovered, but my suggestion concerning ecological or, to use Myers' preferable term, behavioral neoteny, was an attempt to throw light on the matter.

This alarms Myers who is perturbed because, if this is the case, "one or more important changes in the behavioral patterns of cichlids may be involved," and goes on to make some interesting, but to my mind not particularly relevant, observations on the rigidity of breeding behavior patterns in cichlid fishes. Lowe's (1956) observations in Nature have shown that such patterns need not be so rigid as Myers supposes, and surely the inter-generic crosses cited as an example of the persistence of patterns in distantly related forms are in fact indicative of their complete breakdown. It is almost inconceivable to imagine that the same pattern of behavior has persisted throughout the divergent evolutionary history of the fishes to which he refers, particularly as even sibling species of *Drosophila* have been shown to differ in courtship behavior (Manning, 1959), and even mutants of a species may behave in a way different from the normal type (Bastock, 1956).

As to shoaling behavior itself, Myers' emphatic statement that in cichlids the young scatter at the time when the parent-young association breaks down, is quite wrong in the case of some African species, though these need not necessarily be plankton feeders even as juveniles. I am at present studying the biology of the mouth brooding *Tilapia variabilis* Boulenger in Lake Victoria in which species closely-knit shoals of young are a characteristic feature of the areas frequented by these small fishes *after* they have left the parent. This behavior is found also in the substratum spawner *T. zillii* Gervais and certainly in some Nyasan cichlids e.g. *T. saka* Lowe and *T. squamipinnis* Günther (Lowe, 1952). In fact I have earlier (Fryer, 1956) correlated the presence of the so-called Tilapia-mark on the dorsal fin of juveniles of this genus with the shoaling habit. This mark is invariably present in the juveniles and perhaps serves as a visual signal to other members of the shoal. In many species its disappearance coincides with the gradual breakdown of the shoaling tendency. All Myers' remarks about *heterogeneous* shoals being indicative of behavioral neoteny are therefore invalidated. In fact these young cichlids do "lose parent orientation *yet do not scatter*" (Myers' italics) and therefore do break what he calls "an apparently rigidly developed behavioral pattern of cichlids." The moral here is surely that we should not assume that we can formulate laws governing cichlid behavior until far more species have been

studied in nature. As homogeneous shoals do persist after leaving the parent a major objection to my theory of behavioral neoteny is removed.

Incidentally, although Myers is not familiar with any non-shoaling plankton-eating fish, Lake Nyasa can supply examples. Among the clariids of the endemic genus *Bathyclarias* are three which, rather surprisingly, have developed large numbers of long fine gill rakers and have taken to feeding largely on plankton (Jackson, 1959). None of these is known to be a shoaling species. Could it be that these fishes, whose basic adaptations are for a bottom-dwelling mode of life, have adopted what is for a clariid a very atypical habit because the available benthic habitats are few and their ability to adjust themselves to specific niches is less than that of the highly plastic cichlids?

With regard to the permanence of habitats in Lake Nyasa, Myers has not followed my argument correctly. I certainly did *not*, as he claims, compare the permanence of lake environments with the impermanence of stream environments: I compared the permanence of Lake Nyasa with the impermanence of freshwater environments generally whether lacustrine or riverine and specifically emphasized the former. Lakes in general are, in the geological sense, but transient features of the landscape but, as is well known, a few of which Nyasa is one, are more permanent, and it is in these that highly specialized fishes have often evolved. Thus Myers' observations on the behavior of minnows in Texan streams, while interesting, are out of context.

Myers may be on safer ground when he criticizes my acceptance of Larkin's thesis that freshwater fishes in general are relatively unspecialized, and when he points out that my comparison of Nyasan fishes with those of Britain is unfortunate. I admit that this assertion is too sweeping (though by no means without foundation as Larkin (1956), in a well documented account, has already shown, and as I hope to demonstate), but even if I were completely wrong in this respect my main point, namely that many Nyasan fishes are extremely specialized, and that this specialization has promoted allopatric speciation, remains quite unaffected.

It is also relevant to note that the occurrence of 500 or so species of fishes in the Congo system is not unexpected, for this system consists of many rivers with a total length of tens of thousands of miles and embraces a very wide range of habitats. Also similar habitats must be duplicated many times yet be isolated from each other, thereby permitting different species to occupy similar niches in different areas. However, I readily concede that some of the S. American cases cited by Myers must include highly specialized forms.

Two examples illustrate what I imply by "relatively unspecialized" as applied to freshwater fishes. Corbet (in press) has concluded that most of the non-cichlids of Lake Victoria, none of which is the end point of such intralacustrine speciation as characterizes the cichlids of that lake, are facultative feeders—an important manifestation of ecological generalization. Again, in Africa the genus *Barbus* is represented by more than 200 species. These are all very much alike but differ greatly in size, which must permit the exploitation of different niches and the utilization of different foods but, with few exceptions (such as the Nyasan *B. rhoadesii* Boulenger which has developed a wide gape and has become piscivorous) they offer no striking specializations, and many species occur in a wide range of habitats. These are among the tropical fishes of which Myers says "narrow trophic and habitat specializations are the rule," but in fact it is often difficult to see in what ways many species differ *adaptively inter se*.

Lastly Myers suggests that "one of the most promising future lines of attack on the problems of fish evolution in Nyasa would appear to be a careful investigation and comparison of the entire fish fauna and its ecology with that of the Zambesi River, from which the lake was populated." No detailed comparison can be made here nor are sufficient data yet assembled for such a study, but a few already apparent features are so illuminating that they merit brief mention, particularly as they answer Myers who says "If he had compared the specializations of the Nyasan cichlids with those of the fishes of the Zambesi River . . . his comparison would have had some weight." First, however, his statement that the Zambesi fish fauna is large needs correcting. That it is poorly explored is in part true, but recently a series of short expeditions, in one of which I took part, have been made to the Middle Zambesi and, with previous work, have so far revealed in this region (i.e. between the Victoria Falls and the Kebrabassa Rapids) only 29 species of fishes of which one is a migratory eel! (Anon., 1959). Even if the additional species found in the Upper and Lower Zambesi, which are geographically distinct, are added, the total of true freshwater species is not greatly increased. (That a few additional species are known from the Kafue River which is connected to the Zambesi illustrates my comment on the Congo fauna.) The first significant feature of the comparison is now apparent. A long section of the river, which includes several habitats, contains fewer species than can be found regularly on a hundred yards of rocky shore in Lake Nyasa

without counting rare species. In order to survive in the latter community, in which restricted niches have been exploited, a fish must be specialized: in the river such specialization is unnecessary and would in fact, for reasons to be explained, be a liability.

Conditions in the Zambesi vary much throughout the year. At times the river is a raging torrent, in which quiet conditions are to be found only in backwaters and inundated areas: at other times it becomes little more than a series of connected pools in which high temperatures often prevail. I maintain that, with the exception of the local lungfish which can withstand drought by encysting, only generalized fishes can tolerate such changes, and that ecologically specialized fishes, i.e. fishes delicately adjusted to a particular niche, could not do so. A few examples make this clear. Both the Zambesi clariids *Clarias mossambicus* Peters and *Heterobranchus longifilis* Cuvier and Valenciennes, exhibit certain definite specializations such as long sensory barbels and accessory respiratory organs, yet I would regard neither as specialized fishes. Both exist over a wide area under diverse conditions, of which the unstable Zambesi is only one example, both are facultative feeders and virtually omnivorous, and it is difficult to point to any one specialized feature in which they differ adaptively from many of their relatives.

Two of the mormyrids, *Mormyrus longirostris* Peters and *Mormyrops deliciosus* (Leach), besides tolerating the varied conditions imposed by the Zambesi, occur also in the clear waters of Lake Nyasa, in the turbid waters of the quite different Lake Bangweulu, in the Bangweulu swamps which are different again, and in other waters of diverse types. That they are specialized in possessing electric receptors and probably in other ways does not alter the fact that they are ecologically euryvalent.

One of the Zambesi characins, *Alestes imberi* Peters, besides belonging to an anatomically generalized genus (Myers, 1958), is also strikingly generalized in its ecological requirements, is widely distributed and occurs under diverse conditions. In Lake Nyasa its behavior is strikingly different from that of the highly specialized endemic species. It occurs frequently on sandy shores to which, however, it is by no means restricted for, unlike the endemic species of such habitats, it enters affluent streams and sluggish turbid rivers; and, while its endemic associates are often specialized for the collection of particular foods, it is a generalized feeder.

Finally, compare the four cichlids of the Middle Zambesi with the 200 or so now known from Lake Nyasa. These include two widely distributed species of *Tilapia* which, as I have tried to show elsewhere (Fryer, in press) is a generalized cichlid genus, and an as yet unidentified *Haplochromis*. It is generally agreed that the species flocks of *Haplochromis* and allied genera of Lake Nyasa, which include highly specialized species, arose from riverine species of that genus which certainly merit the designation "generalized" on both anatomical and ecological grounds (Trewavas, 1948; Fryer, 1959a).

Without discussing other species enough has been said to show some of the ways in which the fishes of the Zambesi differ ecologically from the endemic forms of Lake Nyasa. In a word they are generalized while many Nyasan endemics are specialized, the latter being the point to which I sought to direct emphasis in my paper.

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GENETICS AND DARWINISM[1]

SPENCER W. BROWN

Department of Genetics, University of California, Berkeley

The symposium on Long Island was undoubtedly one of the most impressive ceremonials in the recent apotheosis of Darwinism. Since professional exigencies will lead most of the readers to the published report, the reviewer will confine himself to brief impressions.

In a symposium on evolution dedicated primarily to genetics, it is astonishing to find that the offerings of the paleontologist play such a primary role, not merely in affording the all important look at the record, but in adducing lessons of basic significance. H. N. Andrews Jr. emphasizes the necessity of avoiding stultification of thought by premature classification, here of an elegantly displayed but incomplete assemblage of early land plants. His philosophy is worthy of extension to the conceptual realm of the evolutionary sciences; who knows what bitter but quite unnecessary controversies are thus to be avoided? For the recent mammals, B. Kurtén makes a bold attempt to relate the rates of simple quantitative changes to the more complex allometric alterations and thereby to the rates of speciation itself; in spite of obvious limitations, the results are admirable. Simpson provided the outstanding contribution of the symposium when he slew the Hydra of supraspecific evolution exposing it as a process presumably identical with that which yields new species but which leads to drastically different results often through the discovery of a new adaptive mode. An immediate application of insight into the process is the realization that each new discovery has its own potential for further evolution; the exploitation of one may be quite unlike that of another. In assessing the results, particularly in coping with small, divergent groups, the taxonomist needs a thorough comprehension of the underlying process.

Of the various hypothetical models of speciation, the one constructed by Carson seems most clearly copied from nature. That such models are themselves models of complexity is illustrated by the ease with which a significant part or vector may be omitted. In his consideration of peripheral populations Carson, leaning perhaps too heavily on Robinson Crusoe cases, forgets the "feeding" function of the center, a hiatus fortunately filled by Levins' remarks in the discussion.

When certain concepts have been worked out through the long and painful process of trial and error, it is a pleasure to see them tested with accuracy and clarity in a new situation; such is the case with Smith-White's well-written analysis of the chromosomal conditions in Australian woody plants. Geographical considerations enable him to state that there was a period of rapid evolution of new chromosome members during the late Cretaceous and early Tertiary followed by stability since the mid-Tertiary.

Although the immediate speciation made possible by the remarkable cytoplasmic factors of the mosquitoes studied by Kitzmiller and Laven is probably not of common occurrence, their work does emphasize the necessity of looking for changes in genetic systems which may lead to rapid exploration of new evolutionary possibilities. The reports of Maxime Lamotte on polymorphism in snails and of Sheppard on mimicry in butterflies are both rewarding summaries of much extensive field work and its critical evaluation. By means of such refined natural history our various models of evolution must eventually be tested.

The introductory and concluding discussions by Mayr, Dobzhansky, and Stebbins provide a background in recent thinking on evolution which is a fitting framework for the twenty-one diverse and individual reports of the symposium.

[1] Cold Spring Harbor Symposia on Quantitative Biology. 1959. Vol. 24. Genetics and twentieth century Darwinism. xvi + 321 pp. The Biological Laboratory, Cold Spring Harbor.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Falconer, D. S. 1960. Introduction to Quantitative Genetics. IX + 365 pp. illus. 35 shillings. Oliver and Boyd. Edinburgh. A textbook of general principles of inheritance of metric traits by a leading investigator of selection in laboratory populations.

Simpson, G. G., Anne Roe and R. C. Lewontin. 1960. Quantitative Zoology. 2nd ed. VII + 440 pp. 64 figs. $8.00 Harcourt, Brace. N. Y. A revision of the 1939 first edition (by the two senior authors) with added material on analysis of variance and other techniques for quantitative treatment of zoological material. Computation instructions based on numerous actually worked examples are provided. This, together with many other features of the book, for example, the emphasis on quantitative treatment of observations rather than on experimental design, make it particularly valuable for much of the readership of this journal.

Sahlins, Marshall D. and Elman R. Service (Eds.). 1960. Evolution and Culture. XII + 131 pp. $3.75. U. Michigan Press. Four essays on cultural evolution by anthropologists from the Universities of Michigan and Oklahoma.

Rensch, Bernhard. 1960. Evolution Above the Species Level. XVII + 419 pp. 113 figs. $10.00. Columbia University Press. English translation of the second edition of the evolutionary classic *Neuere Probleme der Abstammungslehre*.

Tax, Sol (Ed.). 1960. Evolution after Darwin. vol. 1. The Evolution of Life: its Origin, History and Future. VIII + 629 pp. $10.00. vol. 2. The Evolution of Man: Mind, Culture and Society. VIII + 473 pp. $10.00. University of Chicago Press. The first two (out of three) volumes of the proceeding of the University of Chicago centennial celebration of Darwin's *Origin of Species*.

Willmer, E. N. 1960. Cytology and Evolution. IX + 430 pp. illus. $10.00. Academic Press. A text containing a series of essays dealing with the main classes of cells appearing in tissue cultures, and types of cells in different animal tissues and organs, with an emphasis on evolutionary aspects.